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Bionomics of *Culex pipiens pipiens* L., *Culex restuans* Theob., and *Culex salinarius* Coq. (Diptera: Culicidae) in central Iowa

by

John Leslie Shipp

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INTRODUCTION

Mosquitoes are vectors of several arboviruses such as Saint Louis encephalitis (SLE), western equine encephalomyelitis (WEE), eastern equine encephalitis (EEE), and California encephalitis (CE) in North America. These encephalitides can significantly affect the health of humans and animals.

Cases of arthropod-borne encephalitis have occurred in Iowa every year since 1967; the human cases usually involve LaCrosse (LAC) encephalitis which belongs to the CE group (W. A. Rowley, Department of Entomology, Iowa State University, unpublished data, 1980). An epidemic of SLE and WEE occurred in 1975, with 19 and 5 cases, respectively. The number of equine cases of WEE in Iowa yearly exceeds the number of human cases of all the arthropod-borne encephalitides (Dorsey et al., 1978). Two SLE and five WEE virus isolates have been obtained from Culex pipiens pipiens L., Culex pipiens quinquefasciatus Say, Culex restuans Theob., and Culex salinarius Coq. in Iowa from 1971-79 (Rowley et al., 1973; Dorsey et al., 1978; Wong et al., 1978; Rowley et al., 1979; W. A. Rowley, Department of Entomology, Iowa State University, unpublished data, 1980).

Adult females of these Culex species are morphologically similar and are difficult, if not impossible, to identify to species. This is especially the case if scales are missing as is the case in light trap specimens. However, adult males

and 4th-stage larvae can be accurately identified. Recently, electrophoresis was employed to identify adult females of Cx. p. pipiens, Cx. p. quinquefasciatus, Cx. restuans, Cx. salinarius, and Culex territans Walk. (Saul et al., 1977).

The importance of the Culex species (Cx. p. pipiens, Cx. restuans, and Cx. salinarius) to the natural history of SLE and WEE viruses is unknown in Iowa. The sympatric association of these species makes it difficult to attribute a virus isolation to one particular species. Culex p. pipiens is a primary vector of SLE in North America (Luby et al., 1969). However, it is questionable if Cx. p. pipiens transmits arboviruses to humans, since these mosquitoes primarily feed on birds (Murphey et al., 1967; Ekis and Hagmann, 1968; Tempelis, 1975).

It is possible that Cx. restuans and Cx. salinarius function in the natural history of SLE and WEE viruses. Both mosquito species transmit SLE virus, and become infected with WEE virus (Chamberlain et al., 1959; Hayes, 1979). Culex restuans feeds on birds (Murphey et al., 1967; Wright and DeFoliart, 1970). Culex salinarius feeds on humans (LeDuc et al., 1972; Edman, 1974).

Important criteria for determining the vector status of mosquito species are: seasonal abundance; parity of females, and the time period of diapause induction. Some of these factors have been investigated for Cx. p. pipiens

but most have not been studied for Cx. restuans and Cx. salinarius. The objectives of this study were to study the biology of Cx. p. pipiens, Cx. restuans, and Cx. salinarius in central Iowa to determine: (1) seasonal abundance of each species; (2) parity of female population; (3) time period of diapause induction; and (4) if electrophoresis can be used to identify these species.

REVIEW OF LITERATURE

Culex Mosquitoes

Culex genus

The genus *Culex* belongs to the family Culicidae and includes numerous species which occur mostly in tropical and subtropical regions. Taxonomic features of the male terminalia define the subgenera. Three subgenera (*Culex*, *Melanoconium*, and *Neoculex*) occur in Canada and the United States (Carpenter and LaCasse, 1955). This literature review deals only with species in the subgenus *Culex*.

Culex "complex"

The *Culex* "complex" in North America involves *Cx. p. pipiens*, *Cx. p. quinquefasciatus*, and *Culex molestus* Forskal (Barr, 1957). Identification of *Cx. molestus* is based on a biological characteristic, autogeny. Morphologically, *Cx. molestus* is difficult to separate from *Cx. p. pipiens* and *Cx. p. quinquefasciatus*.

Culex pipiens "group"

Several morphologically similar *Culex* species occur in the state of Iowa. Included in this "group" are *Cx. p. pipiens*, *Cx. restuans*, *Cx. salinarius* and possibly *Cx. p. quinquefasciatus* (Rowley et al., 1979). The *Cx. pipiens* "group" differs

from the Culex complex in that Cx. restuans and Cx. salinarius are included in the Culex "group" in Iowa because they are indistinguishable from Cx. p. pipiens and Cx. p. quinquefasciatus on the basis of adult female characteristics. Many difficulties occur in biological studies when it is impossible to differentiate between closely related species. One of the major difficulties is associated with the identification of adult female specimens collected in New Jersey or CDC light traps. The inability to distinguish Cx. p. pipiens from Cx. restuans and Cx. salinarius makes it impossible to study the biology of adults of these species. Also, it is impossible to determine the importance of one or more of these species in the maintenance and natural history of mosquito-borne encephalitis viruses that occur in Iowa.

The Cx. pipiens "group" is important to the natural history of arboviral diseases in North America. Culex p. pipiens and Cx. p. quinquefasciatus are primary vectors of SLE virus (Luby et al., 1969). Culex restuans and Cx. salinarius can transmit SLE virus (Chamberlain et al., 1959). Western equine encephalomyelitis virus has been isolated from Cx. restuans in Manitoba and the eastern United States (Norris, 1946; Hayes, 1979). Also, isolations of EEE virus have been obtained occasionally from Cx. restuans and Cx. salinarius (Casals and Clarke, 1965). La Crosse virus has been

recovered from Cx. p. pipiens and/or Cx. restuans in Wisconsin (Thompson et al., 1972).

Several viruses of no known medical importance have been isolated from Cx. pipiens mosquitoes. Flanders virus has been isolated from Cx. p. pipiens and Cx. restuans, and Turlock virus has been obtained from pools of Culex mosquitoes collected in Iowa (Whitney, 1964; Kokernot et al., 1969; Dorsey et al., 1978). Both Flanders and Turlock viruses are thought to produce harmless infections in birds; neither has been associated with human disease (McLintock and Iversen, 1975).

Both SLE and WEE viruses have mosquito-bird-mosquito transmission cycles (Hess and Hayes, 1967; Luby et al., 1969). Passerine birds, especially house sparrows are important "amplifying" hosts of these viruses. Culex p. pipiens, Cx. restuans, and Cx. salinarius readily feed on passerine birds (Tempelis, 1975). Also, Cx. salinarius may function as a vector in epidemics for the "spill-over" of SLE and WEE viruses to horses or humans since, Cx. salinarius will feed on mammals and birds (LeDuc et al., 1972; Suyemoto et al., 1973; Edman, 1974).

Culex pipiens mosquitoes, in addition to being vectors of several viruses, also have been associated with the disease dog heartworm as a carrier of its agent, Dirofilaria immitis (Hu, 1931; Bemrich and Sandholm, 1966; Seeley and Bickley, 1974).

Natural infections of D. immitis in Cx. p. quinquefasciatus have been reported by Villavaso and Steelman (1970). Experimental infections have been produced in Cx. p. pipiens, Cx. restuans, and Cx. salinarius (Hu, 1931; Bemrick and Sandholm, 1966; Seeley and Bickley, 1974).

Culex pipiens pipiens L.

Classification and geographic distribution

Culex p. pipiens was originally described by Linneaus in 1758, and has had many synonyms (Knight and Stone, 1977). The numerous synonyms reflect the world-wide distribution of this species.

This species occurs in northern Europe and Asia, the southern part of South America, East and South Africa, and North America. Culex p. pipiens occurs primarily from 38 to 53° N latitude in North America (Rempel, 1950; Barr, 1957). In the United States it has been observed below 38° latitude in Alabama, Arkansas, Georgia, Mississippi, North Carolina, Oklahoma, South Carolina, and Tennessee (Carpenter and LaCasse, 1955).

Eggs

The breeding habitats of Cx. p. pipiens include intermittent and permanent bodies of water such as ponds, pools, and artificial containers (Rowe, 1942). Abundant numbers of

this species have been associated with "polluted" water (Horsfall, 1955).

Eggs of Cx. p. pipiens are deposited in well-defined rafts containing approximately 175 eggs per raft (Gerberg et al., 1969). Spielman (1971) reported that anautogenous females could lay as many as 400 eggs per raft. Shroyer and Siverly (1972) found that egg production varied according to the source of the blood-meal. Culex p. pipiens deposited a mean number of 133 and 209 eggs per raft when females fed on guinea pigs and quail, respectively. Some strains of Cx. p. pipiens are autogenous (Spielman, 1971). Egg rafts of autogenous females are considerably smaller than those of anautogenous Cx. p. pipiens. An autogenous egg raft has approximately 70 eggs (Spielman, 1971). Hatching time varies according to temperature. Egg rafts hatch approximately 30 hr after deposition at 26 to 27° C (Gerberg, 1970). At lower temperatures, the incubation period increased. Eggs of a Michigan strain of Cx. p. pipiens did not hatch for 141 hr at 13.5 to 14° C (Horsfall, 1955). During the hatching process, larvae entered the water from the submerged anterior end of eggs (Horsfall, 1955).

Several studies have been conducted to determine what factors enhance the attraction of breeding sites to gravid Cx. p. pipiens. Maw (1970) and Maw and Bracken (1972) found that pools containing the bacteria Pseudomonadaceae and treated

with capric acid and NH_4NO_3 were more attractive ovipositional sites than control pools lacking the bacteria and chemicals. Seemingly, a factor produced by these bacteria was responsible for the attractiveness of the pool. The capric acid and NH_4NO_3 provide nutrients for the growth of bacteria (Maw, 1970).

Larvae

Culex p. pipiens has four larval instars. The development periods of the various instars at 13.5-14° C are: 5 to 6 days, 1st instar; 4 days, 2nd instar; 5 days, 3rd instar; and 9 days, 4th instar (Farid, 1949). The developmental time is much shorter at warmer temperatures. Gerberg (1970) reports a larval developmental period of 6 to 9 days at 27°C. Rowe (1942) reports that the larvae of Cx. p. pipiens are associated with the larvae of Anopheles punctipennis Say, Cx. restuans, and Culex tarsalis Coq.

The morphological characteristics of 4th-stage larvae are such that accurate taxonomic identification of the species is possible. The antennae and siphon tube are the important taxonomic features of 4th-instar larvae. Antennal tufts are large and situated on the outer third of the antennal shaft. The critical taxonomic feature of Cx. p. pipiens is the siphon, which is four to five times as long as wide. Siphonal hair tufts are made up of two to four stout hairs per tuft. Four tufts are present with the second to the last distal tuft

situated lateral to the others (Carpenter and LaCasse, 1955).

Pupae

There is a scarcity of information on the biology of the pupal stage. The developmental period for the pupal stage is approximately 36 hours at 27 °C (Gerberg, 1970). Male pupae are usually smaller and emerge earlier than females (Barr, 1958). The sex ratio of male to female is approximately 1:1 (Clements, 1963).

Adults

Most information concerning the biology of Cx. p. pipiens deals with adults, and aspects of their biology such as mating, swarming, seasonal abundance, flight capabilities, biting activities, host preference, parity, overwintering, and taxonomy. Mating and swarming have been studied by Frohne (1964), and Lea and Evans (1972). Lea and Evans (1972) found that females were not receptive to mating until they were at least 48 hours old. Frohne (1964) studied the swarming and mating behavior of Cx. p. pipiens in the fall in northern Ohio. He concluded that males form mating swarms over trees, buildings and other large objects. Swarming was observed at temperatures as low as 7° C. Maximal swarming activity occurred for 65 minutes at dusk and 30 minutes at dawn. Mating occurred when females entered the swarm.

The seasonal abundance of adult Cx. p. pipiens has been

difficult to establish. Females of this species were most abundant in August at Delaware City and Lewes, Delaware for a 20-year period (Darsie et al., 1953). Seasonal abundance of Culex mosquitoes was observed for a 10-year period at Raynham, Massachusetts by Main et al. (1968). This study grouped Cx. p. pipiens, Cx. restuans, Cx. salinarius, and Uranotaenia sapphirina (O.S.) under the heading "Cx. pipiens type". The peak abundance of females of this group occurred from the latter half of August to mid-September.

Spielman (1971) reported the seasonal abundance of autogenous and anautogenous populations of Cx. p. pipiens for a 3-year period at Boston, Massachusetts. Spielman collected mosquitoes from four isolated sites that were within 310 m of each other. Larvae breeding in the natural sites and from egg rafts oviposited by field-collected females were identified to species. Reared females were tested for autogeny. The anautogenous population reached a maximal level in early August of each year. Increased numbers of autogenous Cx. p. pipiens coincided with declining population levels of anautogenous mosquitoes. The highest peak of autogenous mosquitoes occurred in September.

The seasonal abundance of Cx. p. pipiens has also been determined utilizing artificial pools to collect egg rafts of Culex species near Belleville, Ontario (Maw and Bracken,

1972). Peak abundance of Cx. p. pipiens egg rafts occurred in late August.

Only limited data do exist concerning the flight capabilities of Cx. p. pipiens. MacCreary and Stearns (1937) collected adults in a light trap 13 km offshore of Delaware Bay. Marked males and females have been caught at distances up to 22 km from a release point (Clarke, 1943). Clements (1955) reported that anautogenous females flew a maximum of 5339 m on a flight-mill apparatus.

This species is not normally active during the day but commences biting around dusk. Activity ceases shortly after dark, but biting activity occurs again for a short period around sunrise (Headlee, 1931). As mentioned previously, many investigators have studied the host preference of this species. The data indicate that Cx. p. pipiens is predominantly ornithophilic (Hayes, 1961; Murphey et al., 1967; Means, 1968; Tempelis, 1975). Tempelis et al. (1967) reported that Cx. p. pipiens showed a slight feeding shift from birds to mammals at midsummer in Colorado. This species has also been reported feeding on humans in Boston, Massachusetts and Long Island, New York (Means, 1968; Spielman, 1971). The reasons that Cx. p. pipiens occasionally fed on mammals were not understood.

Parity status (i.e., females that have undergone a

gonotrophic cycle) of Cx. p. pipiens was investigated by Morris and DeFoliart (1971) in Wisconsin. However, both Cx. p. pipiens and Cx. restuans were present in the collections and only 270 females were trapped for the season. Twenty percent of the mosquitoes were parous.

This species overwinters as an adult female. Spielman (1971) suggests that anautogenous Cx. p. pipiens hibernate during winter whereas autogenous populations breed throughout the winter. The hibernaculum of overwintering females includes a diversity of man-made and natural sites. Examples of man-made sites are basements, storm sewers, outbuilding foundations, army bunkers, and mine shafts. Natural sites include caves, escarpments, hollow trees, sink holes, and animal burrows (Jumars et al., 1969; Buffington, 1972; Hayes, 1973).

Investigating factors that affect diapause induction in mosquitoes has generated considerable interest in studies on diapause induction with Cx. p. pipiens. Laboratory studies by Eldridge (1966), and Spielman and Wong (1973b) showed that the first two days of the pupal stage were critical for initiation of ovarian diapause. The most sensitive period was immediately after the larval-pupal molt (Spielman and Wong, 1973b).

A diapause response in Cx. p. pipiens was induced when

mosquitoes were exposed to a photoperiod between 12 hr 45 min and 13 hr 45 min at 18°C (Spielman and Wong, 1973b). Sanburg and Larsen (1973) discovered females entered diapause when the photoperiod was less than 13 hr. The discrepancies between these studies could be associated with experimental design, strain differences of the mosquitoes, or the method of determining diapause-type ovarioles. Sanburg and Larsen (1973) used follicle size, whereas Spielman and Wong (1973b) employed a ratio of length of the follicle to length of the germarium.

Temperature can modify the effect of photoperiod on diapause. Temperature had a reciprocal effect on diapause induction when compared to photoperiod (Eldridge, 1966; Sanburg and Larsen, 1973; Spielman and Wong, 1973b). Only 66% of females had follices of the diapause type when pupal and adult instars were reared at eight hours of light and 22°C (Spielman and Wong, 1973b). At 25°C, females did not enter diapause but 97% of mosquitoes were in diapause at 18°C.

Spielman (1971), and Spielman and Wong (1973a) determined the period of diapause induction of natural populations of Cx. p. pipiens at Boston. These studies indicated that female mosquitoes commenced hypertrophic fat development early in September. Females containing hypertrophic fat deposits were considered in diapause. An inverse relationship exists between the seasonal number of blood-fed mosquitoes and those with fat

body deposits.

Hayes (1973) found the percentage of females with fat bodies doubled in September and increased to 100% by November at McLeansboro, Illinois. Conversely, the percentage of blood-engorged and gravid mosquitoes began to decrease at the beginning of September and essentially reached zero by October.

Generally, it is believed that females entering diapause do not blood feed, but imbibe plant juices. Fat bodies are used to provide energy for survival through the winter (Clements, 1963). Buxton (1935) found that the lipid content of hibernating Cx. p. pipiens decreased about 85% from September to April. Surviving, inseminated females take a blood meal in the spring for egg maturation (Tate and Vincent, 1936).

Eldridge (1966), and Eldridge and Bailey (1979) found that Cx. p. pipiens can undergo gonotrophic dissociation. Gonotrophic dissociation is the failure of ovarian follicles to develop following a complete blood meal. This phenomenon may be important in the overwintering maintenance of certain arboviruses (SLE and WEE). Gonotrophic dissociation occurs when blood-fed mosquitoes are maintained at temperatures below 18°C. At temperatures below 18°C, maturation of the ovarioles does not occur.

Adult Cx. p. pipiens can be easily and accurately identified using structures of the male terminalia. The phallosome is of primary taxonomic significance. It consists of two large sclerotized plates connected at their bases. Each plate has a dorsal and ventral arm. The ventral arm is wing-like and tapered to a point while the dorsal arm is long, slender, straight, and blunt at the tip (Carpenter and LaCasse, 1955).

Culex restuans Theob.

Classification and geographic distribution

Theobald (1901) originally described Cx. restuans from an adult female specimen collected at Toronto, Ontario (Knight and Stone, 1977). This species has been reported in North America from the Gulf of Mexico to Canada essentially from 20 to 53°N latitude (Eldridge et al., 1972).

Eggs

Culex restuans egg rafts are deposited in a variety of breeding habitats. These sites include ditches, pools in streambeds, woodland pools, tree holes, and artificial containers (Carpenter and LaCasse, 1955). Girault (1908) found approximately 260 eggs per raft. Mitchell (1907) reported eggs hatch one to three days after deposition.

Several studies have investigated factors that make a

body of water attractive to gravid Cx. restuans. Maw (1970), and Maw and Bracken (1972) reported that water containing Pseudomonadacae and treated with capric acid and NH_4NO_3 was especially attractive to ovipositing Cx. restuans. These factors were more attractive to Cx. restuans than Cx. p. pipiens. Siverly (1972) found that female Cx. restuans were also attracted by gases, such as methane which is emitted from decaying organic bog soil.

Larvae

Shelton (1973) found that larvae of Cx. restuans develop in water temperatures from 12 to 29°C. Development was faster at the higher temperatures. Complete development required 18 days at 12°C and 4-5 days at 29°C.

Limited information exists on what constitutes the larval diet. Horsfall (1955) found that larval food consisted of organic debris. Ingested strands of algae have also been found in Cx. restuans.

The physical parameters of water containing larvae of Cx. restuans were investigated by Petersen and Chapman (1969), and Williams et al. (1971). Larvae were never collected in water with salinity in excess of 1.25 milliohms. However, Cx. restuans can tolerate a pH range of 3.8 to 8.3.

Rowe (1942) studied the biology of Cx. restuans in Iowa to determine which mosquito species share breeding habitats

with Cx. restuans. The species commonly associated with Cx. restuans are Aedes vexans Meig., An. punctipennis, Cx. apicalis Adams, Cx. p. pipiens, Cx. salinarius, Cx. tarsalis, and Culiseta inornata (Will).

Taxonomic identification of Cx. restuans larvae is easy. The main taxonomic characteristics are the antennae and siphonal hair tufts. Antennae are spiculate, and slightly narrowed and dark beyond the insertion of antennal tuft. The antennal tuft is situated near the middle of shaft. There are three pairs of long single hairs irregularly situated on the siphon. There is also a pair of small subapical tufts that have two or three hairs per tuft (Carpenter and LaCasse, 1955).

Pupae

The developmental period of the pupal stages was investigated by Shelton (1973). The duration of the pupal stage was 7.5 days at 12°C and approximately 2 to 2.5 days at temperatures from 15 to 29°C.

Adults

Studies on the adult biology of Cx. restuans have been concerned with seasonal abundance, flight capabilities, nectar-feeding, host preferences, parity, overwintering, and taxonomy. The seasonal abundance of Cx. restuans has been

studied in various parts of North America employing larval and adult male collections. Maw and Bracken (1972) collected Culex egg rafts deposited in artificial pools and found that the seasonal abundance of Cx. restuans peaked in June and July in Ontario. A similar monitoring method was used at Cedar Key, Florida by Lowe et al. (1974). The seasonal abundance of egg rafts could not be accurately assessed because only 132 rafts were collected for the year. Williams et al. (1971) sampled mosquito larvae in Pocomoke cypress swamp in Maryland from April to November. In these studies, Cx. restuans populations peaked in the spring and again in the late fall.

The seasonal abundance of Cx. restuans has also been studied utilizing male collections. Male mosquitoes were collected using a New Jersey light trap at Belleville, Ontario (Belton and Galloway, 1966). Eighty-eight Cx. restuans were collected with the peak occurring in September and October.

Little is known about the flight capabilities of Cx. restuans. MacCreary and Stearns (1937) collected Cx. restuans at least 5.1 km from the shore of Delaware Bay.

Grimstad and DeFoliart (1975) studied the nectar-feeding behavior of Cx. restuans in Wisconsin and found mosquitoes actively nectar-fed during twilight periods. Swarming behavior was observed in conjunction with nectar-feeding activity.

Many studies have been conducted on the host preferences of mosquitoes. Reports in the literature are in conflict over whether or not Cx. restuans has a preference for birds or mammals. Studies by Hayes (1961) and Means (1968) suggest that Cx. restuans is a general feeder. Bait-trap studies of Murphey et al. (1967), and Wright and DeFoliart (1970), and serological blood-meal identifications (Tempelis, 1975) indicate that this species is an avian feeder. Siverly (1972) reports that species occasionally feeds on man. More research is needed to clarify the host preferences of Cx. restuans.

Investigations of the parity status of this species have been conducted by Morris and DeFoliart (1971) in Wisconsin, and Magnarelli (1975; 1977) in New York. Morris and DeFoliart (1971) also collected Cx. p. pipiens. Results of this investigation were presented earlier for Cx. p. pipiens. Magnarelli (1975) collected mosquitoes using dry ice-baited and unbaited CDC light traps at Danby, New York in 1973. Mosquitoes were trapped from late May to the beginning of September. The seasonal parity rate (i.e., percentage of parous females to the total population examined) was 35%. This was based upon 377 dissected females. Magnarelli (1977) collected 171 female Cx. restuans at Ithaca, New York with a seasonal parity rate of 29%. Only uniparous mosquitoes were detected in this study. Numerous parous females were collected in July,

but the largest percentage of parous mosquitoes occurred in August.

Studies on diapause induction and survival of adult Cx. restuans through the winter have been conducted by Wallis (1959), and Eldrige et al. (1972). Wallis (1959) found that blood-feeding activity of Cx. restuans decreased during late summer and fall. Eldridge et al. (1972) showed experimentally that short photoperiod (8 hr light) decreased blood feeding compared to long photoperiod (16 hr light). The blood-feeding activity of Cx. restuans is also influenced by temperature. Blood feeding activity of females increased under the short photoperiod regime at high temperature (27°C). Eldridge et al. (1972) found that Cx. restuans underwent gonotrophic dissociation when females were exposed to the short photoperiod and temperatures of 15 and 20°C. The effects of photoperiod and temperature on ovarian development in Cx. restuans was investigated by Eldridge et al. (1976). A majority of ovaries in females were in a diapause state at 15°C and 8:16 L:D. In contrast, few ovaries were in diapause stage when mosquitoes were maintained at 25°C and 8:16 L:D. No females were in diapause at 16:8 L:D regardless of the temperature. Therefore, photoperiod exerted the dominant effect on diapause induction in Cx. restuans, but temperature

did have a moderating influence at short photoperiods.

The survival of Cx. restuans through the winter was investigated by Wallis (1959). Blood-fed and sucrose-engorged mosquitoes were placed in separate cages at experimental hibernation conditions to determine which mosquitoes survived winter. Blood-fed mosquitoes sustained high mortality throughout the winter. Only 2% of blood-fed Cx. restuans survived from fall to January, whereas 68% of sucrose-fed females survived the same period of time.

Male Cx. restuans, like Cx. p. pipiens, can be accurately identified according to structure of the external male genitalia. The major taxonomic structure is the phallosome which consists of two large sclerotized plates. Each plate has a long slender apical ventral arm, a short blunt basal dorsal arm which curves outward, and a short triangular tooth situated halfway between the dorsal and ventral arms (Carpenter and LaCasse, 1955).

Culex salinarius Coq.

Classification and geographic distribution

Culex salinarius initially was described from a type specimen collected in New Jersey by Coquillett in 1904. This species has been reported from approximately 22 to 46°N latitude in North America (Eldridge et al., 1972). Linam (1965) found that Cx. salinarius occurs predominantly in the

Atlantic and Gulf Coast regions.

Eggs

Breeding habitats of this species are diversified and have included fresh and foul water, and water with relatively high salinity (Carpenter and LaCasse, 1955; Williams, 1956; Chapman, 1959). Examples of ovipositional sites include grass-bottom pools, ditches, ponds, tree holes, cattle tracks, and artificial containers (Rozeboom, 1942; Carpenter and LaCasse, 1955). Culex salinarius eggs are deposited in rafts in a manner similar to that of Cx. p. pipiens and Cx. restuans. Mitchell (1907) reported that rafts contain 50 to 55 eggs but, Newkirk (1955) found up to 104 eggs in a raft. Andreadis and Hall (1980) found that females lay approximately 170 eggs during the first gonotrophic cycle. The number of eggs per gonotrophic cycle decreased as the number of cycles increased. Shelton (1972) found that a minimum of 0.2 mg of blood (chicken) was required to initiate egg production. One mg of chicken blood produced the largest number of eggs (123). Gerberg (1970) found that at 27°C hatching occurred within three days after oviposition.

Stimuli that affect the attraction of ovipositional sites to gravid Cx. salinarius have been investigated by several researchers. Petersen and Willis (1970) found that Cx. salinarius did not have an universal preference for any

concentration of sodium and chloride salts. Andreadis (1977) found that gravid females showed a preferential response to water associated with developing pupae. The attractant seemed to be a nonfilterable substance of pupal origin.

Larvae

Wallis and Spielman (1953), and Wallis and Whitman (1968) found that larval development required 10 to 15 days at 26.5°C. Shelton (1973) compared larval development at various temperatures (12 to 35°C). Complete development was recorded only from 12 to 29°C. Rudolphs (1926) recovered protozoans (Euglena and Trachelomonas), diatoms, and fungal hyphae from the guts of Cx. salinarius larvae. Culex salinarius larvae can survive a pH range of 4.0 to 8.0 (Petersen and Chapman, 1969; Williams et al., 1971). Petersen and Chapman (1969) reported that electrical conductivity of water containing larvae ranged from 0.35 to 1.07 milliohms.

The larvae of Cx. salinarius are accurately identified by means of the antennae, and siphon tube and tufts. The antennae are constricted beyond the insertion of the antennal tuft. Antennae are pale and spiculate before the tuft but, are dark and less spiculate after the constriction.

The siphon tube is the major taxonomic character. The siphon tube is six to seven times as long as wide. Siphonal tufts are sparse being inserted beyond the pecten teeth with

the subapical tuft situated lateral to other tufts. There are usually four (occasionally five) paired 2- to 4-branched tufts on the siphon (Carpenter and LaCasse, 1955).

Pupae

Shelton (1973) determined that developmental periods for Cx. salinarius pupae were generally similar regardless of water temperature. Approximately 1 to 3.5 days were required for this stage. Adult emergence was maximal at 23°C over a temperature range of 12 to 35°C.

Adults

Adult biology of Cx. salinarius has been more extensively studied than the other stages of this species. Mating, seasonal abundance, flight capabilities, biting activity, host preferences, parity, diapause, and taxonomy of Cx. salinarius have been studied.

Mating behavior has been observed by Wallis and Spielman (1953) who recorded that mating by colonized Cx. salinarius was brief, lasting only a few seconds. Swarming was not observed in the cages.

Seasonal abundance of Cx. salinarius has been monitored using larval and adult abundance. Lowe et al. (1974) employed artificial pools for collecting egg rafts in Florida but collected just 17 rafts for the year. Williams et al. (1971) found that the majority of larvae of Cx. salinarius

were collected in July in their study at Pocomoke cypress swamp.

The peak abundance of adult females was in July and August at Delaware City and Lewes, Delaware over a 20-year period (Darsie et al., 1953). October was a peak period for females in Hancock County, Mississippi over a period of four years (Harden and Poolson, 1969).

There were only limited data available on the flight capabilities of Cx. salinarius. MacCreary and Stearns (1937) reported collecting adults at least 12.8 km into Delaware Bay in light traps.

Murphey and Darsie (1962) found that the active period for Cx. salinarius was from sunset to sunrise. Maximal numbers of adults were collected using New Jersey light traps during the sixth 15-minute period following sunset. The third 15-minute period was the peak period for human biting activity.

Animal bait-trap studies have shown that this species feeds readily upon mammals or birds (Hayes, 1961; Murphey et al., 1967; Wright and DeFoliart, 1970). A majority of the serological studies suggests that Cx. salinarius feeds predominantly on mammals (Edman and Downe, 1964; Schaefer and Steelman, 1969; LeDuc et al., 1972; Suyemoto et al., 1973; Edman, 1974).

Feldlaufer and Crans (1979) compared the relative

attractiveness of carbon dioxide to nulliparous and parous mosquitoes collected with a New Jersey light trap. Mosquitoes were collected from 6 June to 15 September. Twenty-four and 35% of the Cx. salinarius collected by carbon-dioxide-baited and unbaited traps, respectively, were parous.

Eldridge et al. (1972), and Eldridge et al. (1976) investigated diapause induction in Cx. salinarius and found that a temperature of 15°C decreased blood-feeding activity at 8:16 and 16:8 L:D. There was no significant difference in blood-feeding activity between photoperiods at 20 and 27°C. Follicular development was also not influenced by photoperiod. Culex salinarius ovaries were not in a diapause state when the photoperiod and temperature were 8:16 or 16:8 L:D at 25°C. At 15°C, 30 to 50% of the ovaries of females exposed to either photoperiod were of the diapause-type. Temperature seemed to exert a dominant influence on diapause induction in Cx. salinarius.

Accurate identification of Cx. salinarius to species is possible based on the structure of male phallosome. The dorsal arm is a stout pointed structure bending medially at a right angle. The stout bluntly-pointed ventral arm has a single projection on its inner margin, and a series of teeth midway between the apices of the dorsal and ventral arms (Carpenter and LaCasse, 1955).

Electrophoresis as a Means of Species Identification

Some species of medically important insects are morphologically indistinguishable in the adult stage, or if indistinguishable, the taxonomic characteristics are not always present. Examples of mosquitoes in this category are the Anopheles gambiae group, Aedes hendersoni Cockerell - Aedes triseriatus (Say), and several Culex species.

Researchers have investigated the possibility of applying biochemical differences between species as a taxonomic "tool". Initial studies were conducted with free amino acids and employed various biochemical procedures to detect differences between species.

The detection of specific biochemical differences between mosquito species with electrophoresis has only been employed since the late 1960s. Researchers investigated changes in isozyme patterns of enzymes to detect differences between species. The early work of Chen (1967) demonstrated differences in hemolymph proteins of autogenous Cx. molestus and anautogenous Cx. p. quinquefasciatus Say employing disc electrophoresis. An extensive survey of nonspecific esterase patterns in 14 species and strains of mosquitoes was conducted by Freyvogel et al. (1968). Esterase patterns varied with the species. Trebatoski and Haynes (1969) investigated isozyme patterns of 12 species of mosquitoes. These

researchers concluded that electrophoresis may be applied to the problems of mosquito systematics and evolution. Other general surveys utilizing electrophoresis have resulted in similar conclusions (Warren and Breland, 1969; McDonald et al., 1972).

Igbokwe and Downe (1978) investigated differential electrophoretic patterns among strains of Aedes aegypti L. This study showed that differences between general protein staining patterns existed between strains. Also, there was a greater divergence in the electrophoretic patterns of females than in males.

A biochemical key to adult members of the An. gambiae group has been formulated with data generated by electrophoresis (Miles, 1979). This key was based on genotype frequencies associated with the distances that the isozymes migrated in electrophoretic gels. Ayala and Powell (1972) concluded a gene locus detected by presence of an isozyme was diagnostic only if an individual was correctly assigned to one of two or more species with at least 99% probability.

Since the work of Chen (1967), several other studies have investigated the application of electrophoresis for the identification of Culex species. Kimura et al. (1971) undertook a study on analysis of Cx. p. pipiens, Cx. p. pallens Coq., and Cx. p. quinquefasciatus, utilizing disc

electrophoresis. No characteristic differences were found between the three species with the egg and pupal extracts. Their analysis was based on general protein staining. Disc electrophoresis was again applied to determine if differences exist between three European strains of Cx. p. pipiens (Schumann, 1973). No difference was found with the three species, but sexual dimorphism existed between adults with the number of detectable protein fractions.

In North America, Cupp and Ibrahim (1973) found differences between Cx. p. pipiens, Cx. p. molestus, and Cx. p. quinquefasciatus employing immunoelectrophoresis. Saul et al. (1977) also demonstrated differences in isozyme patterns of Cx. p. pipiens, Cx. p. quinquefasciatus, Cx. restuans, Cx. salinarius, and Culex territans Walk. with electrophoresis. Differences in the zymogram patterns for the species were detected by staining for isozymes of aldehyde dehydrogenase.

MATERIALS AND METHODS

Description of Study Site

The study site was located at Ames, Iowa at the junction of U.S. Highways 30 and 69. The site was situated on the properties of both the Iowa Conservation Commission Nursery and the Ames City Water Pollution Control Plant. Sewage ponds and natural drainage from fields formed a small meandering stream that flowed eastward into the Skunk River. A deciduous woodlot of black walnut, ash, cottonwood, maple, oak, and hackberry trees occurred in the study area. Herbage was primarily jewel weed, Solomon's seal, Virginia waterleaf, stinging "nettles", and birds.

Wildlife was abundant in the area, especially cottontail rabbits. Other mammals present included raccoons, squirrels, deer, and opposums (Pinger, 1974). The predominant birds of the area were pheasants, woodpeckers, robins, cardinals, grackles, brown thrashers, house sparrows, swallows, and mourning doves.

Description of Trapping Methods and Trapping Procedure

Studies were conducted in 1978 to 1979 at two locations at opposite ends of the woodlot in the study site. Trapping methods included dry ice-baited CDC light traps, suction traps, resting boxes, and artificial pools in 1978. Three

resting boxes and one trap of each of the other methods were operated at each location. Precipitation, and maximum and minimum air temperatures were recorded daily at the Water Pollution Control Plant.

The resting box was an opened-end cube with a flaired extension (46 x 30 x 30 cm) attached to the open end. The unit was painted flat black.[1] A cotton bag with a drawstring was inserted in the box to collect resting mosquitoes. The color of the bag in the box was red, while the extension of the bag attached to the flaired opening was black. The resting boxes were placed 18 m inside the woods and spaced 22 m apart along a transect.

Suction and CDC light traps were placed at the perimeter of the woods at least 27 m apart. The suction trap was a New Jersey light trap without the light and canopy. The trap was painted flat black with a black cotton cloth attached to the bottom to cover a CDC collection bag.

Artificial pools were two-ring wading pools with an inside diameter and depth of 107 and 20 cm, respectively. Pools were covered with 4-mil black polyethylene film and the bottom was lined with sod. Pools were filled with well water. The water level was maintained near the top of the pool.

Two additional trapping methods were employed in 1979. A New Jersey light trap was operated at each location and

[1]Pactra Imperial, Pactra Industries, Los Angeles, California.

sweeping was conducted with a standard 38 cm diameter insect net along a 75 m path at each area. In addition, a second artificial pool was added at each site. The extra methods and traps were included in 1979 to verify results obtained in 1978.

Mosquitoes were trapped at each location from May to October. Mosquitoes were transported alive to the laboratory and immobilized at -70°C. Frozen mosquitoes were placed on a cold table for sorting to sex and identified to species with the aid of a dissecting microscope. Mosquitoes were kept frozen to prevent deterioration of tissues that were used in future experiments.

Female Culex mosquitoes were placed in 8-ml stoppered vials. The vials were sealed with black electrical tape and placed at -70°C. Adult male Culex were placed in Petri dishes and maintained at room temperature until identified. Both vials and petri dishes were labelled according to trapping date, location, and trapping method employed to collect the specimens.

All egg rafts were collected from the artificial pools in 1978. Rafts were transported to the laboratory in Petri dishes containing water-saturated pieces of filter paper. Individual egg rafts were placed in 515 ml Dow^{R} plastic containers. The larvae were maintained at 26±2°C and fed TetraMin^{R} fish food. Identification of the species that

deposited the egg raft was based on identifying 4th- stage larvae from that raft. A minimum of 25% of the rafts collected per pool was identified in 1978. A maximum of 25 egg rafts was collected per pool and identified in each trapping period in 1979.

Species Identification

Identifying adult female Culex mosquitoes of the Cx. pipiens "group" to species is almost impossible. To overcome this problem, Culex mosquitoes were identified on characteristics of the adult male terminalia and 4th-stage larvae. Male Culex were identified according to the structure of the dorsal and ventral arms of the phallosome (Ross and Horsfall, 1965). The terminalia were cleared and mounted on microscope slides. The external genitalia plus two or three abdominal segments were clipped from the abdomen of the mosquito. The terminalia were cleared by placing them in a crucible of boiling 10% potassium hydroxide (KOH) for 10 minutes. Terminalia were removed and the clearing process (KOH) neutralized by putting the mosquitoes in a dish of 1% acetic acid solution. Next, the terminalia were placed in a dish of 70% alcohol for 5 minutes. The genitalia were situated ventral side-up in Permount^{R} or Turtox^{R} mounting medium on a labelled 75 x 25 mm microscope slide. Circular

18 mm cover slips were placed over the specimens after the mounting medium was allowed to harden for 1 to 2 hr. A maximum of 20 mosquitoes was placed on a slide. Mosquitoes were identified to species with the aid of a compound microscope at 100X magnification. Terminalia of all male Culex were identified in 1978 while a maximum of 75 males per trap per week were identified in 1979.

Fourth-stage larvae reared from egg rafts deposited in the artificial pools were also identified to species. The rearing procedure for the larvae was described in the previous section. Mosquito larvae were killed in hot water and placed in a small amount of water in a watch glass dish. The larvae were examined with the aid of a dissecting microscope at 40X magnification. A minimum of four 4th-stage larvae were checked per egg raft. The criteria employed for species identification were the length of the siphon tube, and number of hairs and distribution of siphonal tufts (Carpenter and LaCasse, 1955; Stojanovich, 1961).

Parity in Culex Mosquitoes

Parity determinations were made on adult female Culex collected in 1978 and 1979. The mosquitoes were trapped weekly with dry ice-baited CDC light traps, New Jersey light traps, suction traps, and sweeping. Mosquitoes were frozen

at -70°C in vials that were labelled according to trapping method, location, and trapping date.

Ovaries were dissected from the abdomen of a mosquito for determination of gonotrophic age of a female. This was accomplished by placing the adult female on a microscope slide with two drops of distilled water. The ovaries were removed by teasing the last two abdominal segments away from the remainder of the abdomen. The ovaries were dissected with the aid of a dissecting microscope at 10X magnification using probes consisting of applicator sticks with minuten nadelns attached to one end. One ovary was placed on a dry labelled microscope slide and the other ovary was placed in a Stenner dish containing Aedes physiological saline (Appendix).

Two methods were employed to determine the parity status of females. Detinova's method of ovarian tracheation was used to determine whether or not the female was parous (Detinova, 1962). This procedure involved examining the air-dried ovaries on the microscope slides. The ovaries were examined with a compound microscope at 200X magnification. An ovary was considered nulliparous (i.e., not having undergone a gonotrophic cycle) if the tracheoles on the ovary were in tightly coiled skeins. Uncoiled skeins of tracheoles were indicative of a parous ovary.

If the initially examined ovary was parous, the other

ovary was checked to determine the number of gonotrophic cycles that female had undergone. The dilation method of Podovodova as elaborated on by Detinova (1962) was employed for determining the number of gonotrophic cycles. Individual ovarioles were dissected from the ovary. This was accomplished by dissecting the ovary on a microscope slide containing one drop of crystal violet-saturated sodium chloride solution (3:1). This solution relaxed the ovarioles for easier desiccation and also stained the ovariole for rapid identification of dilatations. Ovarioles were teased apart using minuten probes described earlier. Dissection was accomplished with the aid of a dissecting microscope at 60X magnification. A 18-mm circular coverslip was placed over the dissected ovarioles to prevent desiccation of the ovarioles. Ovarioles were examined using a phase contrast microscope at 200 to 500X magnification.

The number of gonotrophic cycles was determined by counting the number of dilations on the pedicel of the ovariole. Dilatations consisted of remanents of nurse cells and follicular epithelium. Each dilatation represented a single gonotrophic cycle. A minimum of six ovarioles were examined to discern the parity status of each ovary.

Gravid mosquitoes were classified as parous but, the number of gonotrophic cycles could not be determined.

Dilatations on the pedicel were not visible with a mosquito containing mature eggs. Twenty-five mosquitoes per trapping method per week from each location were examined for parity.

Diapause in Culex Mosquitoes

Determinations of whether or not mosquitoes were in diapause were made for adult female Culex collected in 1978 and 1979. The mosquitoes were trapped weekly from May to October with dry ice-baited CDC light traps, New Jersey light traps, suction traps, and sweeping. Female Culex were sorted on a cold table and placed in vials corresponding to the trapping date, location, and trapping method employed. Two drops of water were placed in each vial to prevent desiccation of mosquitoes. The vials were stored at -70°C until the females were examined.

For examination, a female was put on a microscope slide that was placed on the stage of a dissecting microscope. Light was reflected with the mirror up through the specimen. The necessary structures were detected with the light using 30X magnification to define if a female was in diapause.

Two criteria were employed to determine whether or not a female was in diapause. The major criterion involved the deposition of fat bodies containing oil globules in the abdomens of mosquitoes. This criterion was based on

observations of females collected and examined in the fall of 1978. This method was only applied to Culex mosquitoes collected in 1979.

The other criterion used to classify the period of diapause induction involved the seasonal abundance of gravid Culex. This method was an indirect assessment of diapause in a mosquito population since gravid females indicate recent blood-feeding activity. This criterion was applied to mosquitoes collected in 1978 and 1979.

Polyacrylamide-gel Disc Electrophoresis

Polyacrylamide-gel disc electrophoresis was used to evaluate the electrophoretic identification of proteins of Cx. p. pipiens, Cx. restuans, and Cx. salinarius. Mosquitoes involved in the tests were 1st generation progeny from field-collected egg rafts.

Individual egg rafts were placed in Dow^{R} plastic containers. Larvae were fed daily two to three drops of a slurry of TetraMin^{R} fish food. Larvae were fed from 1st instar until pupation.

Egg rafts were identified by examining the 4th-stage larvae, after which larvae of the same species were grouped together. Approximately 500 4th-stage larvae were placed in white enamel pans (25 x 42 x 7 cm) for pupation. Pupae

were placed in one-pint ice-cream cartons. The species name and the date of emergence of adults were labelled on the carton. Adults were provided daily access to 0.30M sucrose-saturated cotton pledgets for nutrition. Larvae were reared and adults were maintained at 26.5 $\pm$ 1°C and 80 $\pm$ 5% RH.

Both nulliparous and gravid females of each species were tested electrophoretically for species identification. Nulliparous mosquitoes were 5 to 6 days old when tested. The gravid females were blood fed when 5 to 6 days old and tested when the mosquitoes were 9 to 10 days old. Nulliparous and gravid mosquitoes were of different ages because time was required for the digestion of the blood meal and the maturation of eggs. All females were frozen at -70°C when the various age classes were reached to prevent denaturation of the enzyme. Females were frozen a maximum of 16 days prior to testing with electrophoresis.

Polyacrylamide-gel disc electrophoresis was the electrophoretic procedure employed in the study. Aldehyde dehydrogenase enzyme (EC 1.2.1.3) was the enzyme that was examined. An anionic gel system was used with a tris 0.04 M-glycine 0.04 M electrode buffer (pH 8.8 to 9.1). The electrode buffer was maintained at 5°C and pH was determined on the cold buffer. A liter of fresh buffer was prepared for each electrophoretic test.

Electrophoretic separation of the sample was accomplished in a polyacryalmide gel. The gel was divided into two components (upper and lower gels). The major difference between the two gels was the pore size (molecular "sieving" effect) of the gel. The pore size was based on the percent of acrylamide in the gel. This was calculated by dividing the volume ratio of the acrylamide component by the sum of the volume ratios of the various components constituting the gel. This number was divided into the quantity of acrylamide that was used in the gel to give the percent of acrylamide. The pore size of the upper and lower gels was 2.5 and 7.5%, respectively. The components and mixing ratios for the upper and lower gels are presented in Table 1.

Prior to a test, gel tubes, 5 mm in diameter (I.D.) and 75 mm in length, were placed in the polymerization rack (Figure 1). A hollow rubber cap was placed over one end of gel tube before it was placed in the polymerization rack. A 1.0 ml syringe was used to apply 0.9 ml of the lower gel solution to the gel tubes to ensure that the exact amount of gel was placed in the tubes. After a couple tubes were filled in this manner, a 3.0 ml syringe was used to fill the remainder of the tubes to level of the first tubes. Tubes were tapped gently to remove trapped air bubbles. A couple drops of water were layered on top of gel solution with a

Table 1. Anionic gel system

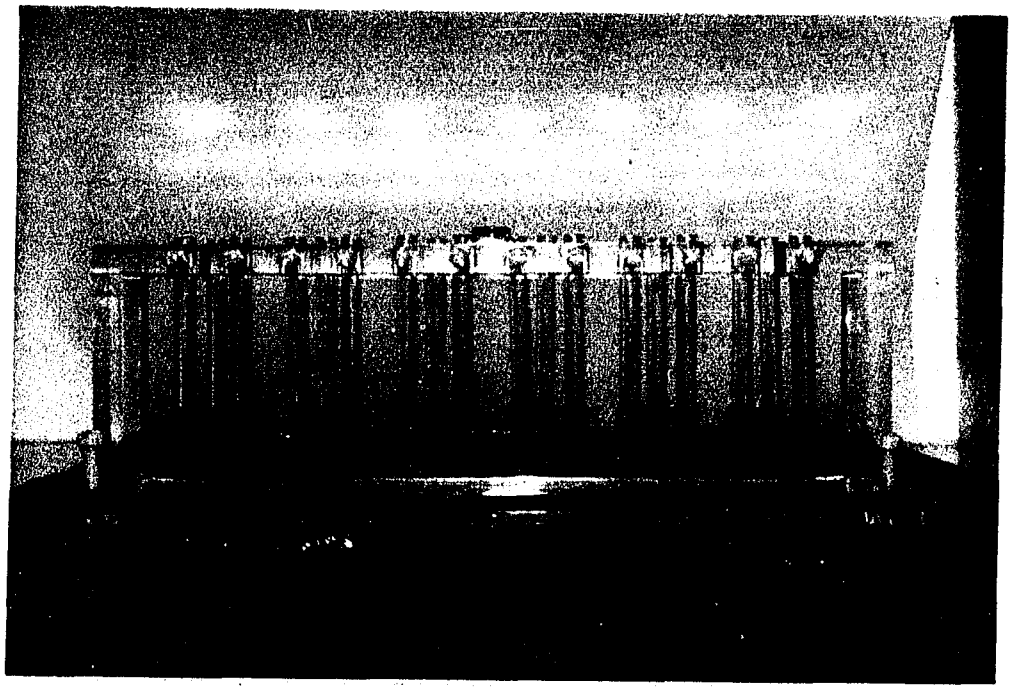
Volume ratios	Components/100 ml distilled water	Amount
Lower gel		
1	Acrylamide	30.00 g
	Bisacrylamide	0.80 g
	Water to volume	
1	Tris	18.15 g
	1N HCl	24.00 ml
	Temed	0.24 ml
	Water to volume	
2	Ammonium persulfate[a,b]	
	Water to volume	
Upper gel		
1	Acrylamide	10.00 g
	Bisacrylamide	0.80 g
	Water to volume	
1	Tris	2.23 g
	1M H_3PO_4	12.80 ml
	Temed	0.10 ml
	Water to volume	
1	Riboflavin	
	Water to volume	2.00 mg
1	Ammonium persulfate[a,b]	0.07 g
	Water to volume	

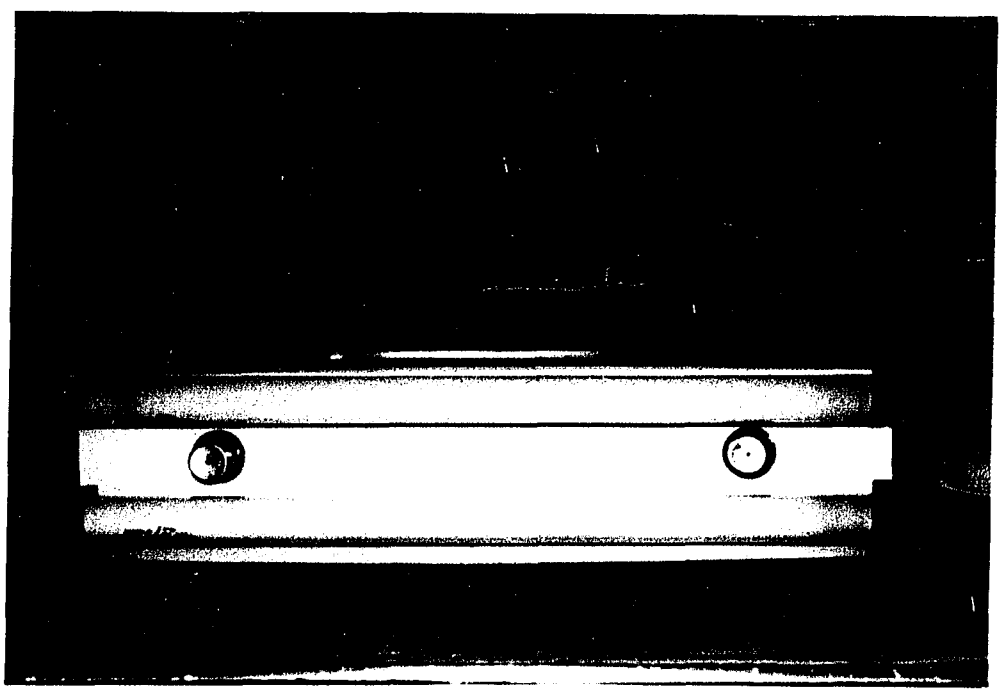
[a]Add this component last to the gel solution.

[b]Stock solution of this component is good only for a week.

Figure 1. Electrophoretic gel tubes situated in the polymerization rack

Figure 2. Fluorescent light source





1.0-ml syringe. Care was exercised not to mix the water and gel solution. Lower-gel solutions were placed in front of a fluorescent light for 30 minutes to polymerize (Figure 2).

Water on top of the gels was absorbed with a Kimwipe® after the gels had solidified. A 1.0 ml syringe was used to apply 0.3 ml of upper gel solution to each tube. Water was layered on top of upper gel and the gel was polymerized for 15 minutes, after which the water was removed.

Test samples of nulliparous and gravid mosquitoes were prepared while the upper gel was solidifying. Eighteen samples were tested with each electrophoretic "run". A sample involved only a single adult female mosquito. The individual mosquito was ground in 40 µl of tris 0.15 M - 1N HCl buffer (pH 9.5) (homogenizing buffer) in 5 ml capacity Bellco® [1] tissue grinder. The quantity of the components used in the homogenizing buffer are presented in the lower gel section of Table 1. Homogenizing buffer was applied to the grinding tubes with Finnpipette®. Both the buffer and the tissue grinders were maintained constantly in wet ice. Mosquitoes were homogenized as completely as possible and centrifuged in the tissue grinders at 5800 x G at 4°C for 5 minutes. Twenty microliters of supernatant of each sample was applied to the top of the upper gel with the Finnpipette.®

[1] Bellco Glass Inc., Vineland, New Jersey.

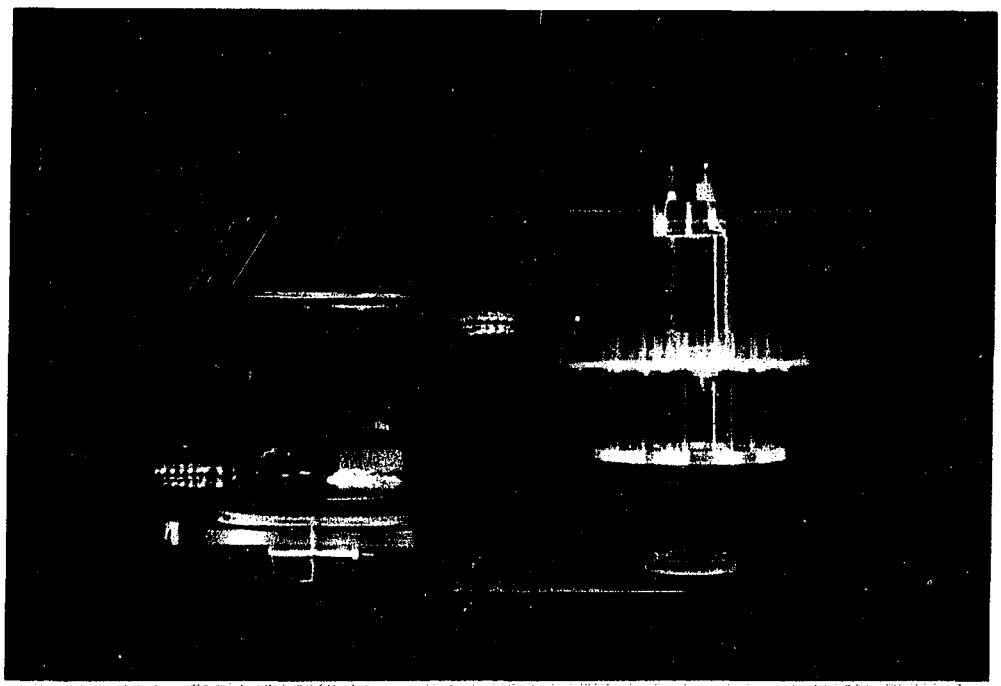
Gel tubes were removed from the polymerization rack and dipped halfway in electrode buffer before passing the tube through the rubber grommet-lined openings in the upper chamber of the disc electrophoresis apparatus (Figure 3). Tubes were arranged so part of the upper gel was visible in the upper chamber. A drop of electrode buffer was placed on the bottom tip of the gel tubes to prevent air bubbles from forming.

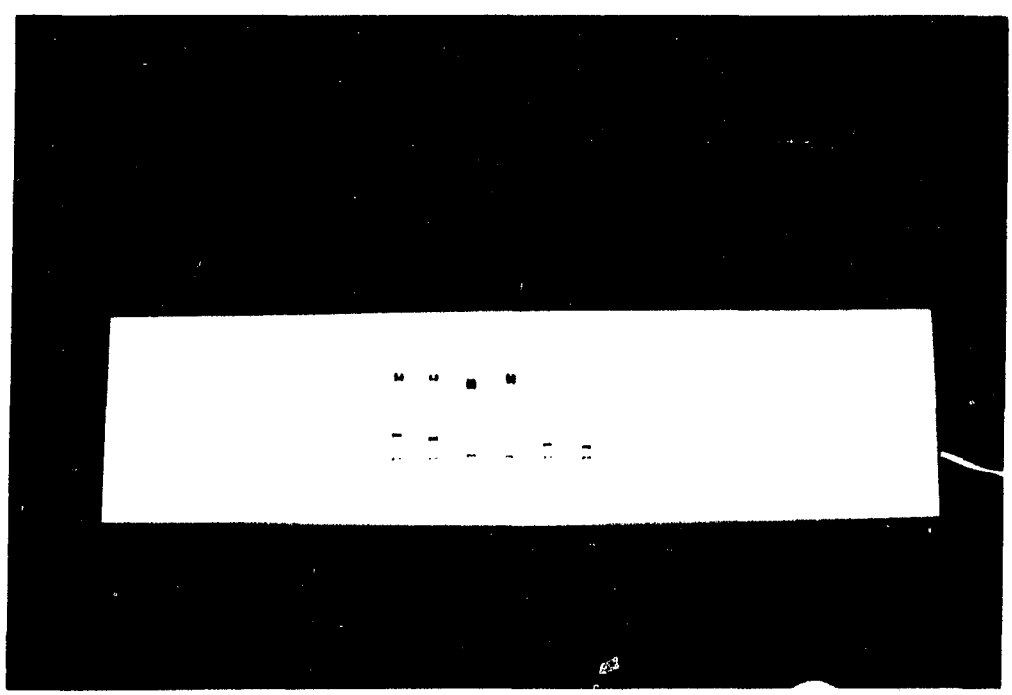
The lower chamber of the disc apparatus (Figure 3) was filled with the electrode buffer after which the upper chamber was situated on top. A drop of bromophenol blue - 25% sucrose solution (indicator dye) was carefully layered on the sample with a Pasteur pipette. Approximately 2 mg of bromophenol blue dye was added to 5 ml of sucrose solution to prepare the indicator-dye solution. The remainder of electrode buffer was added to the upper chamber. Electrophoresis was conducted for 62 to 90 min at 2 ma per tube at 5°C until the indicator dye had migrated to the end of at least one gel tube.

Gels were removed from the tubes by squeezing water between the outside of gel and inner surface of the glass with a 21-gauge needle attached to a 20 ml syringe. Gels were individually placed in a staining solution in 17 x 100 mm Falcon tubes^{R} and incubated at 37°C for 60 min. Staining solution contained 1 ml acetaldehyde, 15 mg NAD, 10 mg Nitro

Figure 3. Upper and lower disc-electrophoresis chambers

Figure 4. Stained gel situated over the fluorescent light source





blue tetrazolium, 2-mg Phenazine methosulfate, and 2-mg Medola blue in 100 ml tris 0.10 M - HCl 0.10 M buffer (pH 8.55). Phenazine methosulfate and Meldola blue were added to the staining solution because Saul et al. (1977) found that Medola blue stains a protein not detected with the other dye.

Staining patterns were evaluated immediately after incubation. Gels were placed on a white opaque plastic cover over a fluorescent light source (Figure 4). The distances that the indicator dye and protein bands migrated in the gel were recorded. Staining intensity of the bands was also noted. Gels were fixed and stored in 7% acetic acid solution after the readings.

RESULTS AND DISCUSSION

Seasonal Abundance of *Culex* Mosquitoes

The seasonal abundance of *Cx. p. pipiens*, *Cx. restuans*, and *Cx. salinarius* was determined by collecting adult males and monitoring egg rafts laid by female mosquitoes throughout the season. The two different evaluation methods were employed for each species to determine seasonal distribution and the number of broods for a season. A brood is defined as all the individuals emerging at approximately one time period from eggs laid by a group of females (Torre-Bueno, 1973).

The number of each species collected each year is presented in Table 2. Three thousand seven hundred and ninety adult males and egg rafts of the three species were collected in 1978. In 1979, 5,563 *Culex* mosquitoes (males and egg rafts) were collected.

Table 2. Number of adult male *Culex* and egg rafts collected at Ames, Iowa, 1978 and 1979

	1978		1979	
	Males	Egg rafts	Males	Egg rafts
Culex pipiens pipiens	956	498	3606	105
Culex restuans	18	2267	425	685
Culex salinarius	4	47	269	473

The seasonal abundance of Cx. p. pipiens was determined in 1978 and 1979, utilizing both methods. Figure 5 shows population levels of adult males for both years. The peak abundance of this species occurred in late summer (August and September). There were three broods of Cx. p. pipiens in 1978 and 1979 as indicated by Figure 5. The initial peak of adult males was progeny of overwintering females. The last two broods (especially the last one) contribute to the potential overwintering population. More males were collected in 1979 than in 1978 because of the New Jersey light trap and sweeping methods in 1979. Both methods together collected 2984 out of 3606 Cx. p. pipiens males trapped in 1979.

The seasonal abundance of this species was determined also by the number of egg rafts deposited in artificial pools. The patterns of seasonal abundance of Cx. p. pipiens based on egg raft collections are illustrated in Figure 6. The initial peaks in 1978 and 1979 represent egg rafts deposited by overwintering females. The number of Cx. p. pipiens broods indicated by egg rafts was different each year. There were four broods in 1978 and three in 1979.

A discrepancy exists in population data when Cx. p. pipiens males and egg rafts are used for determining the seasonal abundance. A major ovipositional peak occurred at approximately the same time that the abundance peak of males

Figure 5. Seasonal abundance of Culex pipiens pipiens L. based on adult male collections at Ames, Iowa

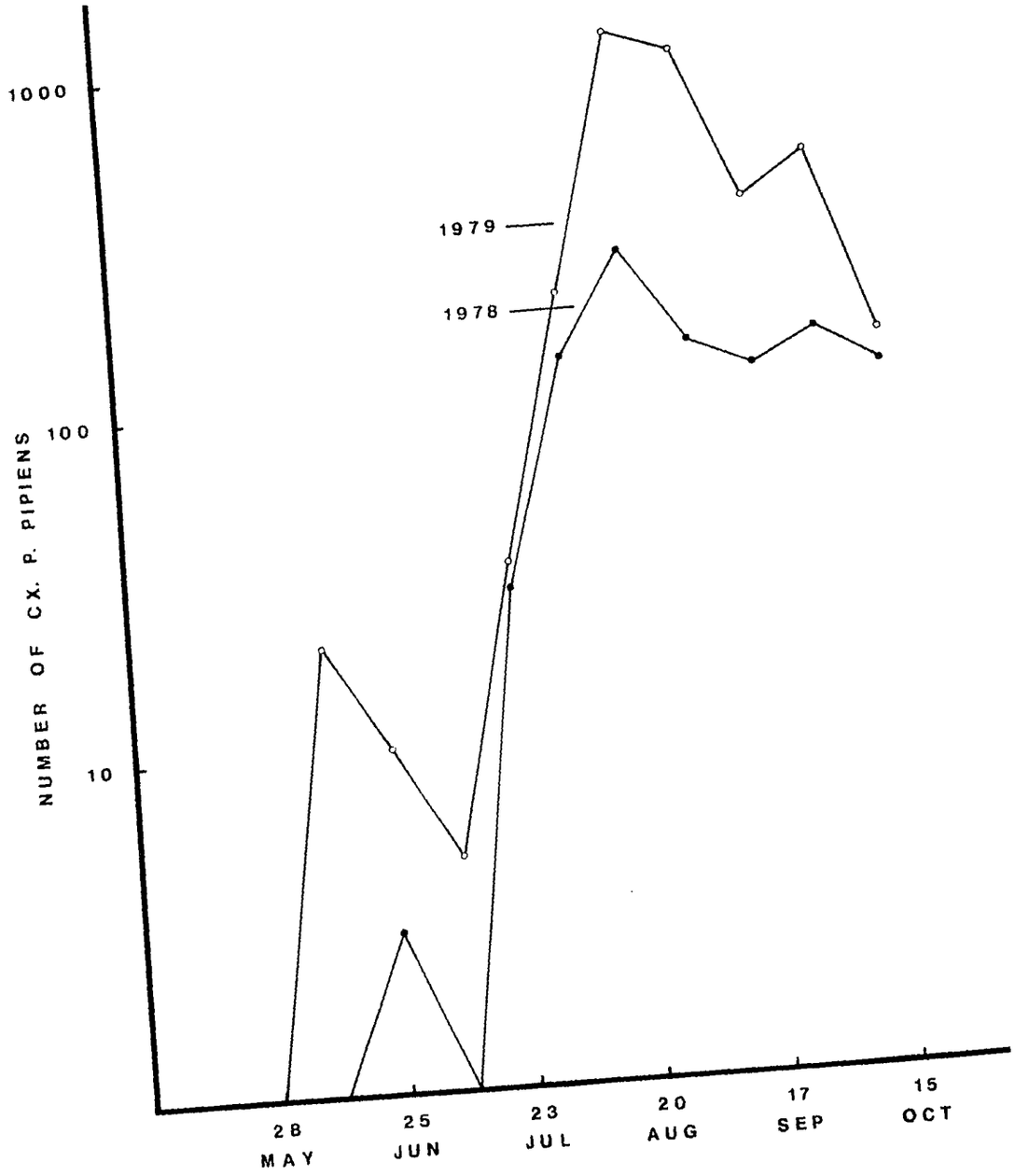
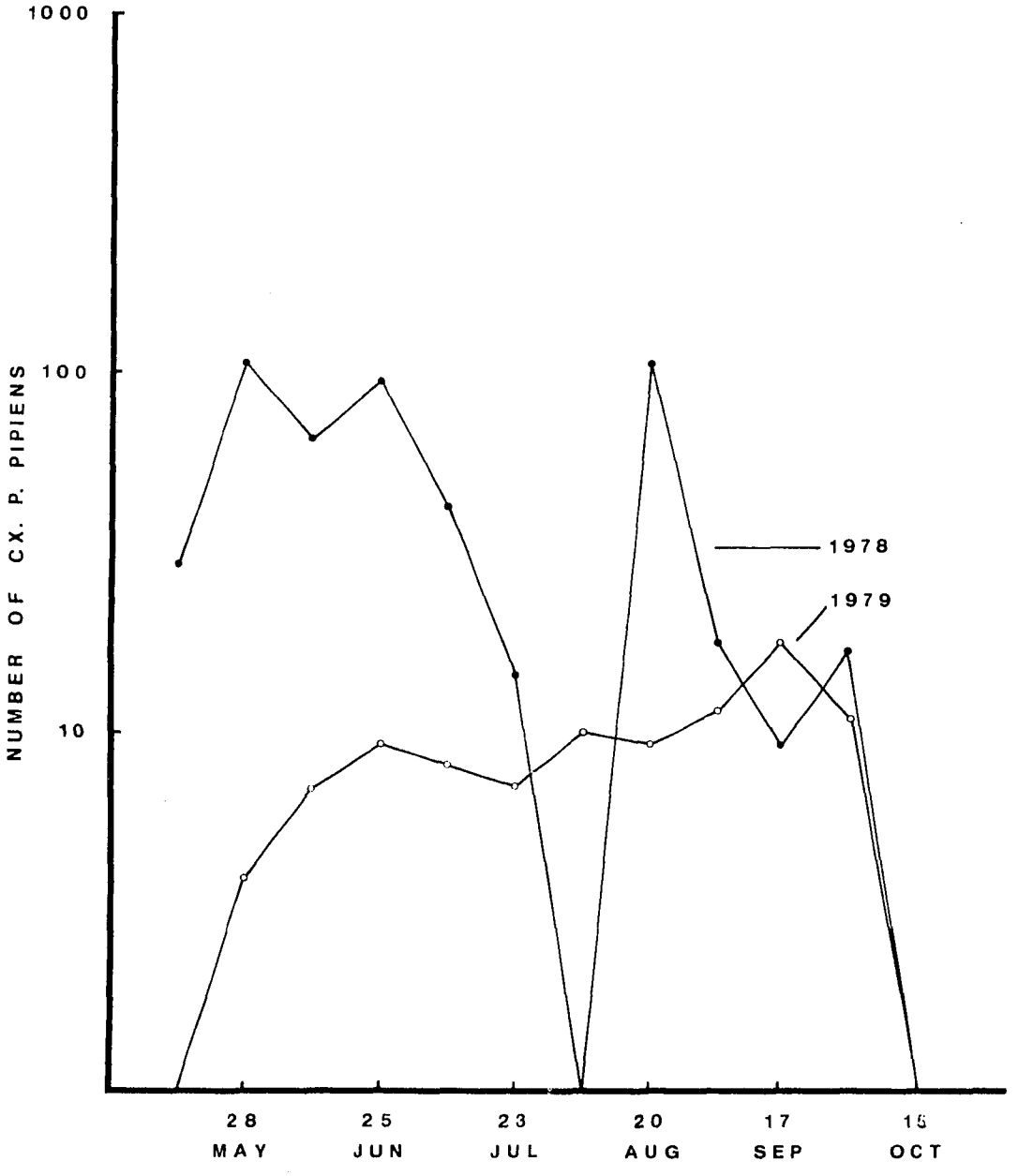

1000
100
10
NUMBER OF CX. P. PIPIENS
1979
1978
28 MAY
25 JUN
23 JUL
20 AUG
17 SEP
15 OCT

Figure 6. Seasonal abundance of Culex pipiens pipiens L. based on egg raft collections at Ames, Iowa


1000
100
10
NUMBER OF CX. P. PIPIENS
1978
1979
28
MAY
25
JUN
23
JUL
20
AUG
17
SEP
15
OCT

was found both years. However, there was also a peak of oviposition activity in June in 1978. Only a few males were collected in the corresponding period of time. Possibly, the difference in the seasonal patterns using the two methods was related to the absence of New Jersey light traps and sweeping in 1978. This method resulted in fewer males being collected in 1978. There was not a difference between the patterns in 1979. The New Jersey light trap and sweeping methods seemed to be crucial to evaluating the seasonal abundance of male Cx. p. pipiens. The seasonal abundance of this species in Iowa was similar to that found in previous studies (Darsie et al., 1953; Main et al., 1968; Spielman, 1971; Maw and Bracken, 1972).

The seasonal abundance of Cx. restuans in 1978 and 1979 was determined using the same methods. The patterns of seasonal abundance of adult males are presented in Figure 7. Males were found only in the early fall of 1978, but population peaks occurred in June and early fall in 1979. New Jersey light traps and sweeping account for the differences between the two years. There was a single brood in 1978; however, there were four broods in 1979.

The seasonal abundance of Cx. restuans as determined by egg raft collections is illustrated in Figure 8. The majority of the egg rafts were deposited from late May to the beginning of July in 1978 and from mid-May to late July in

Figure 7. Seasonal abundance of Culex restuans Theob. based on adult male collections at Ames, Iowa

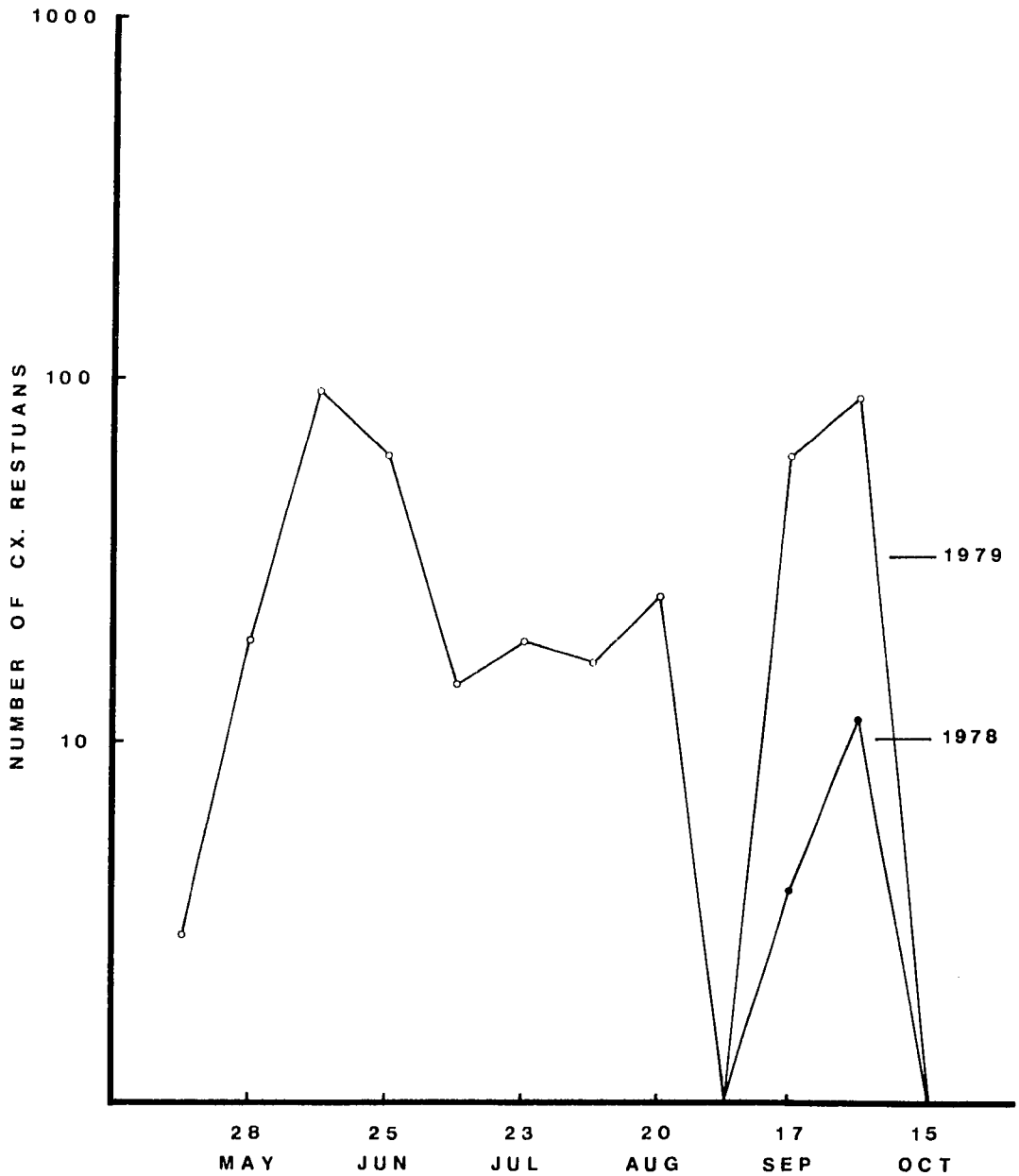
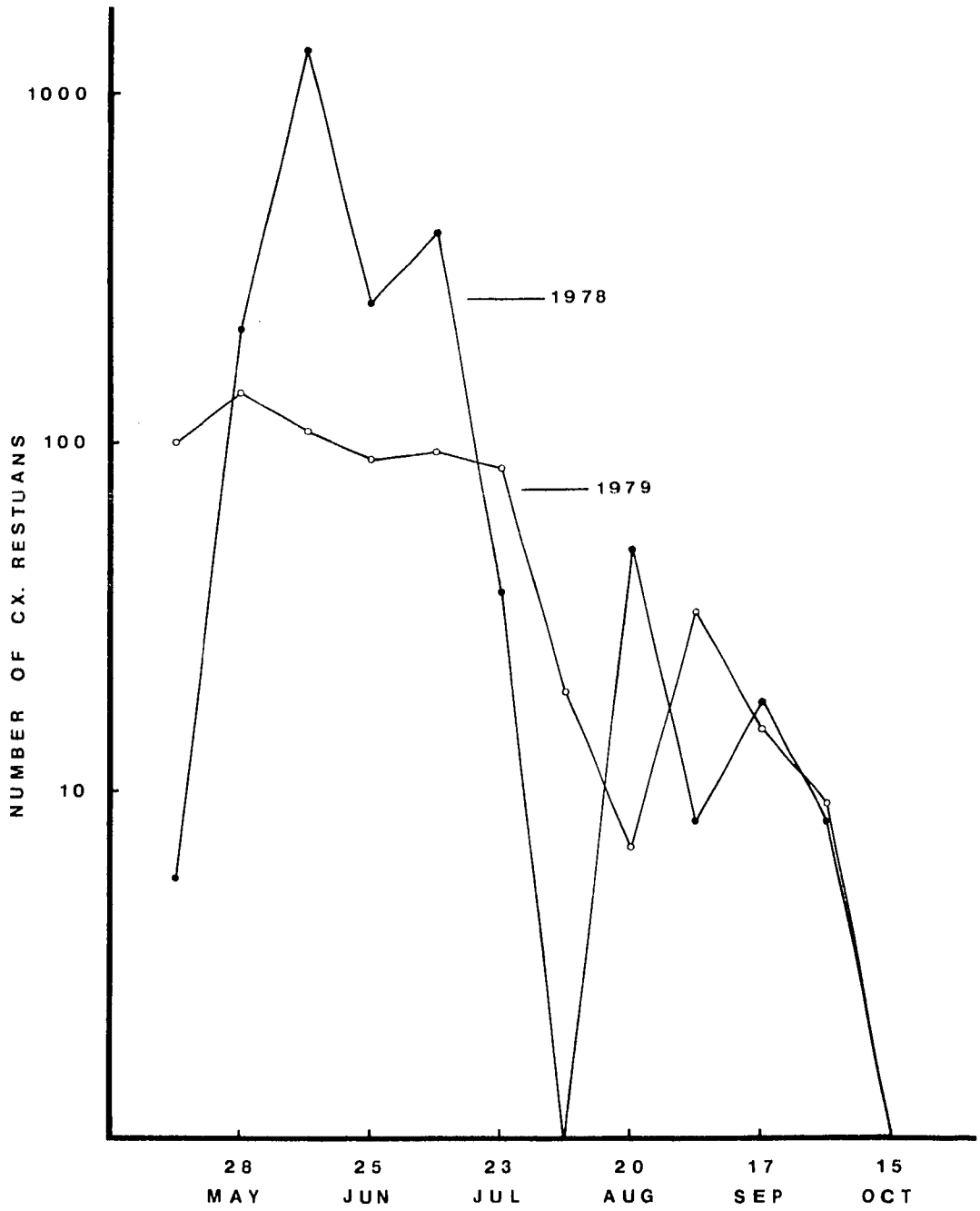

1000
100
10
NUMBER OF CX. RESTUANS
1979
1978
28 MAY
25 JUN
23 JUL
20 AUG
17 SEP
15 OCT

Figure 8. Seasonal abundance of Culex restuans Theob. based on egg raft collections at Ames, Iowa


1000
100
10
NUMBER OF CX. RESTUANS
1978
1979
28
MAY
25
JUN
23
JUL
20
AUG
17
SEP
15
OCT

1979. There were four and three broods, respectively, in 1978 and 1979.

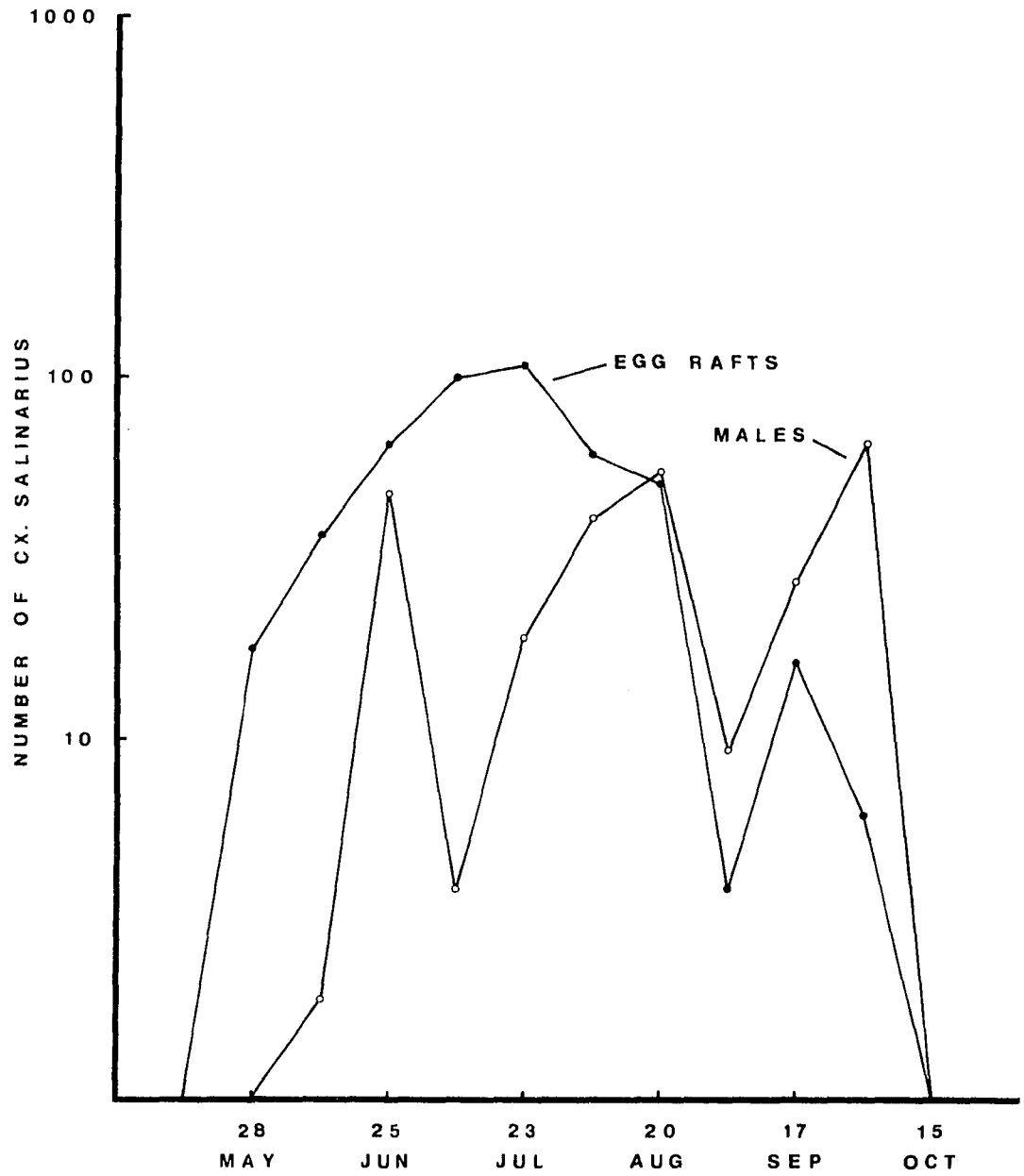
Males were detected in the early fall in 1978 whereas the egg raft method indicated that females were present from May to October. In 1979, the seasonal abundance levels determined by both male and egg raft collections were similar. Population peaks based on male collections lagged only a week behind population peaks represented by egg raft collections.

Maw and Bracken (1972) found that the majority of gravid Cx. restuans occurred in June and July. Williams et al. (1971) found a peak abundance of larvae in the spring and again in early fall. These periods correspond to the times that most of the male Cx. restuans were collected in this study.

The seasonal abundance of Cx. salinarius was evaluated only in 1979 (Figure 9). Three adult male broods were present for the season. The abundance level was similar for all three population peaks. Peak ovipositional activity of Cx. salinarius occurred from June through mid-August (Figure 9). However, some ovipositional activity occurred in mid-September.

The seasonal abundance of Cx. salinarius is different when the two methods are compared. Male collections indicate that there were three broods, all were approximately the same

Figure 9. Seasonal abundance of Culex salinarius Coq. at Ames, Iowa, 1979


1000
100
10
NUMBER OF CX. SALINARIUS
EGG RAFTS
MALES
28
MAY
25
JUN
23
JUL
20
AUG
17
SEP
15
OCT

size and occurred about three to four weeks apart. Only two broods could be determined by the egg raft method, and most egg rafts were associated with the first brood. The seasonal peak of Cx. salinarius occurred in July and August. Darsie et al. (1953) also reported peak abundance of Cx. salinarius in July and August.

The periods of peak seasonal abundance of Cx. p. pipiens, Cx. restuans, and Cx. salinarius were different. Culex restuans occurred predominantly in the spring and early summer. The peak abundance of Cx. salinarius occurred mid-summer while most of Cx. p. pipiens were trapped in late summer and early fall.

The different times of the year that peak abundance of each species occurs may affect the role each species plays in the natural history of SLE and WEE viruses in Iowa. The initial phase in the natural history is an amplification stage. This involves a build-up of virus in susceptible vertebrate hosts and vector mosquito populations. Suitable "amplifying" hosts for SLE and WEE viruses are passerine birds (Hess and Hayes, 1967; Luby et al., 1969). Tempelis (1975) reported that Cx. restuans readily feed on passerine birds. Culex restuans is the most abundant of the three mosquito species in the spring and early summer. Chamberlain et al. (1959), found this species transmits SLE virus. Thus, Cx. restuans may serve as an important mosquito vector for

amplification of these arboviruses.

Epidemics or epizootics of SLE and WEE occur in late summer (McGowan et al., 1973; Rowley et al., 1979). The conditions for disease outbreaks include high virus activity in natural hosts and vectors, available susceptible hosts, and abundant mosquito vectors. Both "normal and aberrant" hosts are involved in an encephalitis outbreak. "Aberrant" hosts are "dead-end" hosts because of a low virus titer in the blood of these hosts (Casals and Clarke, 1965; Clarke and Casals, 1965). A high virus level must occur in the blood of a host to establish virus replication in the cells of the salivary gland of mosquitoes. The presence of virus in the salivary gland enables a mosquito to transmit the virus to a susceptible host.

If different hosts, "normal and aberrant", are involved in epidemics and epizootics, mosquito species must be present that feed on both types of hosts. Culex p. pipiens and Cx. salinarius fulfill these requirements. Peak abundance of Cx. p. pipiens occurs in late summer and early fall. Culex p. pipiens can blood feed on infected "normal" hosts vectoring SLE virus (Luby et al., 1969).

Culex salinarius is a mosquito vector that could transmit the virus from "normal" to "aberrant" hosts. This mosquito species feeds on both birds and mammals (Edman, 1974; Tempelis, 1975; Cupp and Stokes, 1976). The peak

abundance of Cx. salinarius coincides with occurrences of epidemics of SLE and WEE, and epizootics of WEE (McGowan et al., 1973; Rowley et al., 1979). Also, Cx. salinarius transmits SLE virus (Chamberlain et al., 1959).

Another aspect of the seasonal abundance of these three species deals with mosquito populations measured by the two methods. More male Cx. p. pipiens were collected than egg rafts. This phenomenon occurred both years (Table 2). In contrast, Table 2 also shows that more egg rafts of Cx. restuans and Cx. salinarius were collected than adult males. These results may be interpreted two ways. Little is known concerning the biology of male mosquitoes. My data show that New Jersey light traps and sweeping collected numerous male Cx. p. pipiens, but not many of the other two species. Possible reasons for this may be associated with the longevity and resting habitats of males. The chance of collecting Cx. restuans and Cx. salinarius decreases if the survival period of these species is shorter than that of Cx. p. pipiens. Also, Cx. restuans and Cx. salinarius males may not rest in the wooded areas, especially at ground level, where sweeping was conducted. Lastly, male Cx. p. pipiens may be more attracted to light than the other species.

A second interpretation of the differences between the number of males and egg rafts involves the ovipositional

behavior of each species. Natural ovipositional sites were available in the area. Numerous larvae of Cx. p. pipiens were collected from the sewage ponds. No larvae of Cx. restuans and only a few Cx. salinarius were identified from larvae collected in these ponds.

The reason for differences in the breeding habits of these species is unknown. It is significant that Cx. restuans and Cx. salinarius readily deposit egg rafts in small bodies of water. This indicates that populations of these species can be established in areas where only temporary water is found. Examples of such areas would be urban regions that have potential container-breeding habitats or in woodlots containing tree holes. Establishment of populations of Cx. restuans and Cx. salinarius in urban regions could introduce SLE virus into the area. These differences in the breeding habits are important when considering the use of artificial pools for arboviral ovipositional traps (Surgeoner and Helson, 1978). Gravid Cx. restuans and Cx. salinarius are attracted to artificial pools. However, in this study, only a few Cx. p. pipiens oviposited in these pools. The use of trapping methods that collect mosquitoes attracted to artificial pools may bias surveillance monitoring of SLE virus activity. Virus activity may be underestimated if Cx. p. pipiens are not collected.

Parity Studies in *Culex* Mosquitoes

Parity is defined as the condition of an adult female that has undergone a gonotrophic cycle or contains mature eggs. Parity rate refers to the proportion of parous mosquitoes to the total female population. The number of female *Culex* mosquitoes (*Cx. p. pipiens*, *Cx. restuans*, and *Cx. salinarius*) collected in 1978 and 1979 was 4,363 and 14,235, respectively. Seasonal parity rates ranged from 19% in 1978 to 47% in 1979. The pattern of seasonal abundance of parous *Culex* was similar both years (Figures 10 and 11).

The seasonal parity rate was highest in the spring each year. This phenomenon is attributed to several things. Overwintering female *Culex* mosquitoes survive the winter as inseminated adult females. In the spring, females leave their hibernating sites to nectar-feed and ultimately blood-feed. The blood meal provides the stimulus and nutrients for egg maturation. Overwintering females that deposit eggs comprise the initial spring *Culex* population. This population has a high parity rate.

Another factor influencing parity rate in the spring is host availability. *Culex p. pipiens* and *Cx. restuans* are mainly avian blood-feeders (Murphey et al., 1967; Tempelis, 1975). The availability of birds is greater during this time of year because of the breeding activity of birds

Figure 10. Seasonal abundance of female Culex mosquitoes at Ames, Iowa, 1978

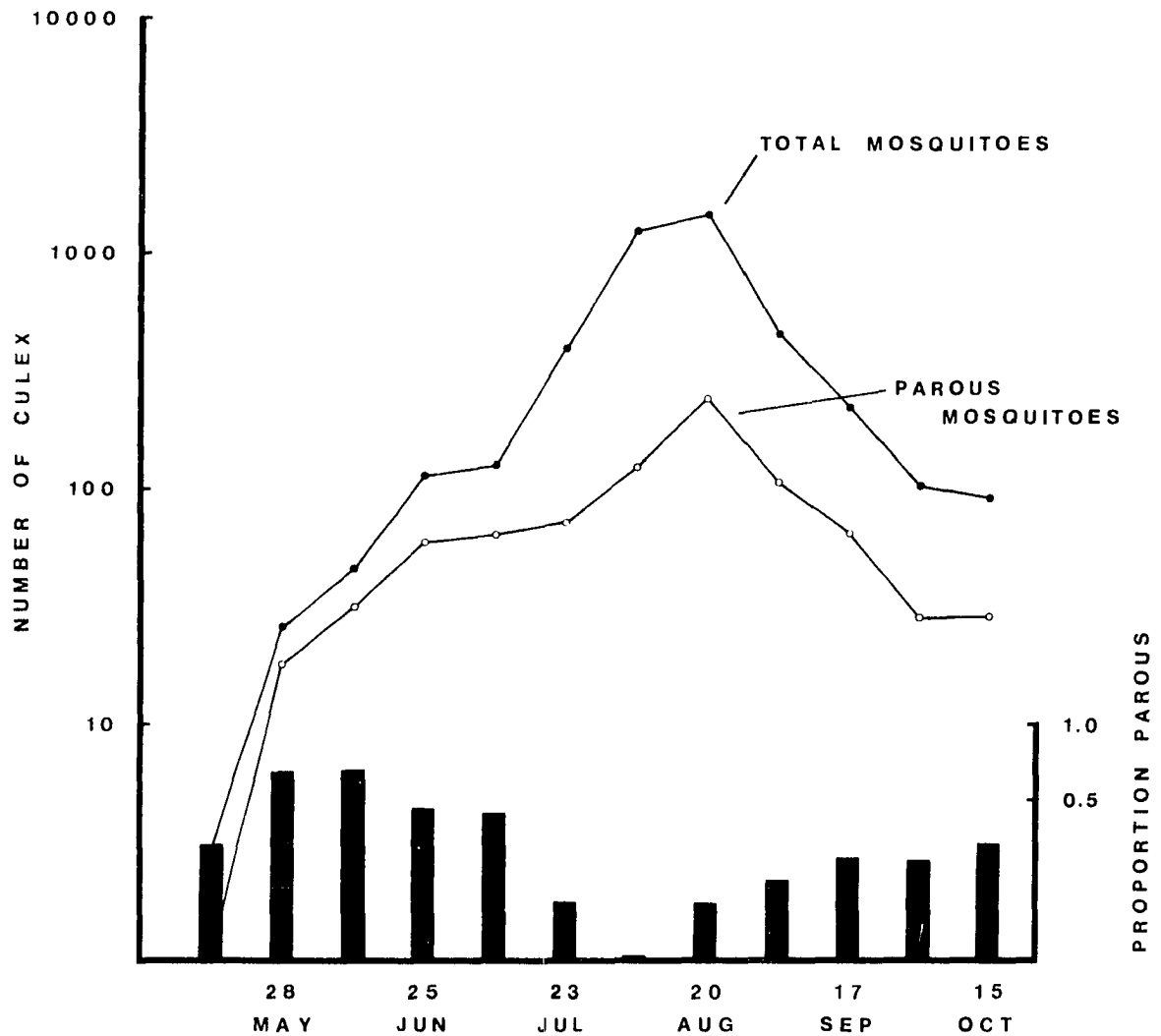
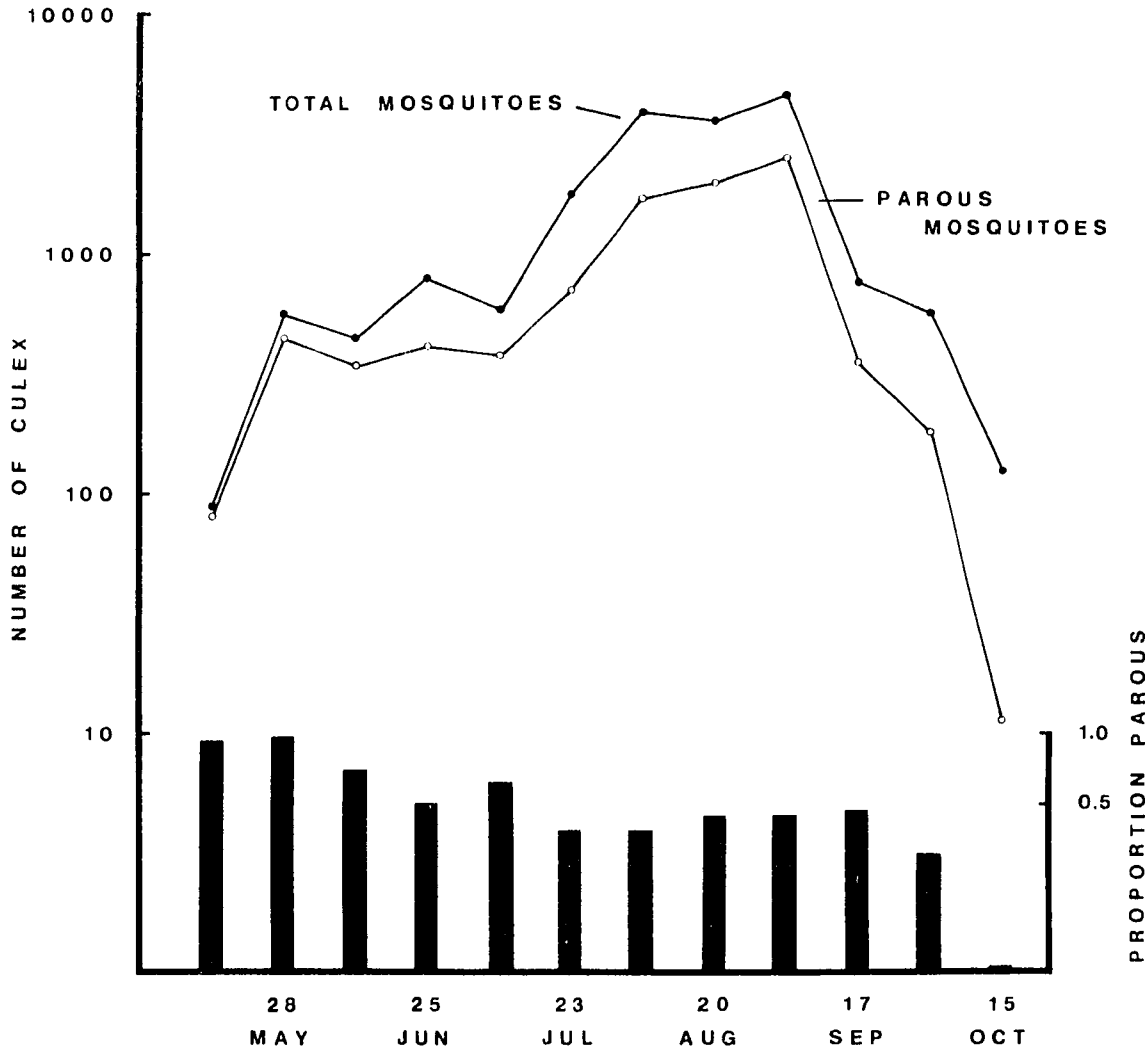

10000
1000
100
10
NUMBER OF CULEX
TOTAL MOSQUITOES
PAROUS
MOSQUITOES
1.0
0.5
PROPORTION PAROUS
28
MAY
25
JUN
23
JUL
20
AUG
17
SEP
15
OCT

Figure 11. Seasonal abundance of female Culex mosquitoes at Ames, Iowa, 1979


10000
1000
100
10
NUMBER OF CULEX
TOTAL MOSQUITOES
PAROUS MOSQUITOES
1.0
0.5
PROPORTION PAROUS
28 MAY
25 JUN
23 JUL
20 AUG
17 SEP
15 OCT

(Johnsgard, 1979; Stokes, 1979). Also, nestling birds have fewer defenses against biting mosquitoes. The plumage on young birds is sparse, and the movements of the nestlings are restricted. Blackmore and Dow (1958) conducted a study with Cx. tarsalis to determine differential feeding of female mosquitoes on nestling and adult birds of the same species. Culex tarsalis were more successful in blood-feeding on nestlings than adult birds.

A third reason for a high proportion of parous Culex in the spring is the presence of numerous breeding sites. Approximately 7 to 8 in of rain fell both years in May and June. The heavy rainfalls created many ovipositional sites.

The parity rate of the Culex mosquitoes was lower in late July and August. The largest number of parous mosquitoes was concomitant with the population peak of the total Culex population (Figures 10 and 11). The result was a lower parity rate since the number of parous females was low compared to total number of Culex mosquitoes.

Some adult female Culex underwent more than one gonotrophic cycle both years. The peak abundance of these females occurred in August. Ten percent of the parous Culex collected in August of 1979 were biparous or triparous. Only 5% of the mosquitoes were biparous during the corresponding period in 1978. The greater percentage of biparous and triparous mosquitoes in 1979 could possibly be associated with

the higher number of Cx. salinarius that year.

Parity is related to the role these Culex species play in the natural history of SLE and WEE viruses in Iowa. Mosquitoes are infected and transmit arboviruses by blood feeding. Anautogenous females need a blood meal to develop mature eggs while autogenous mosquitoes do not. All the Culex species were anautogenous at the study site. Parous mosquitoes in this study had deposited eggs or contained mature eggs. Therefore, parity is an indication of a previous blood meal.

In spring, amplification of arboviruses occurs in natural hosts. A good "amplifying" vector (Culex restuans) is abundant during this time period. The parity rate was high in the spring for the three Culex species. This high parity rate indicates that these Culex species are potential "amplifying" vectors since the amount of blood-feeding activity of the total Culex population was high.

Seasonal abundance data show that Cx. restuans is the most abundant species in spring (Figures 6, 8, and 9). Culex restuans can transmit SLE virus (Chamberlain et al., 1959). Also, WEE virus has been isolated from this species (Norris, 1946; Hayes, 1979). The results of these studies indicate that Cx. restuans may play a crucial role in amplifying SLE and WEE viruses in the spring in Iowa.

Numerous parous Culex occurred in August both years. Culex p. pipiens and Cx. salinarius were the most abundant species at this time (Figures 5, 7, and 9). Both species have been shown to transmit SLE virus (Chamberlain et al., 1959). Also, WEE virus has been isolated from Cx. pipiens "group" in Iowa (Rowley et al., 1973; Dorsey et al., 1978). The importance of these two mosquito species to the natural history of SLE and WEE viruses seemed to be that these mosquitoes have populations that peak at the time of year when virus is transmitted from natural "amplifying" hosts to other susceptible hosts (i.e., horses and humans).

Diapause in Culex Mosquitoes

Diapause is an important adaptive mechanism for survival through unfavorable environmental conditions such as low winter temperatures. Beck (1980) defines diapause as a genetically determined state of suppressed development. Environmental parameters may control initiation of this state.

Parity in adult mosquitoes is closely associated with diapause. Blackmore and Dow (1962), and Hudson (1978) found that mosquitoes in diapause are generally nulliparous. Wallis (1959) demonstrated that nulliparous Cx. restuans survive winter conditions whereas parous mosquitoes do not

survive the same situation. In the present study, 2 of 167 females in diapause were parous. Both of these mosquitoes were uniparous. Blackmore and Dow (1962) found only a few parous Cx. tarsalis overwintering in mines in Colorado. They reported 2 parous mosquitoes out of 753 specimens. Jumars et al. (1969) found that 36% of diapausing Cx. p. pipiens were parous during winter in Delaware. However, autogeny was demonstrated in this population and only 5% of the parous mosquitoes were multiparous. Autogenous females do not require a blood meal for egg maturation. Thus, only 5% of mosquitoes had taken a minimum of one blood meal.

The interest in whether or not parous mosquitoes overwinter successfully is related to the idea that parous adults could provide a mechanism for survival of arboviruses during the winter. On only three occasions have SLE and WEE viruses been isolated from mosquitoes collected in the winter (Blackmore and Winn, 1956; Bailey et al., 1978). It seems that the overwintering of arboviruses by parous mosquitoes in diapause is not an important overwintering survival mechanism for these viruses.

The period of diapause induction was determined directly with fat body deposits and indirectly by the pattern of seasonal abundance of gravid females in this study. Culex females with hypertrophic fat development containing oil

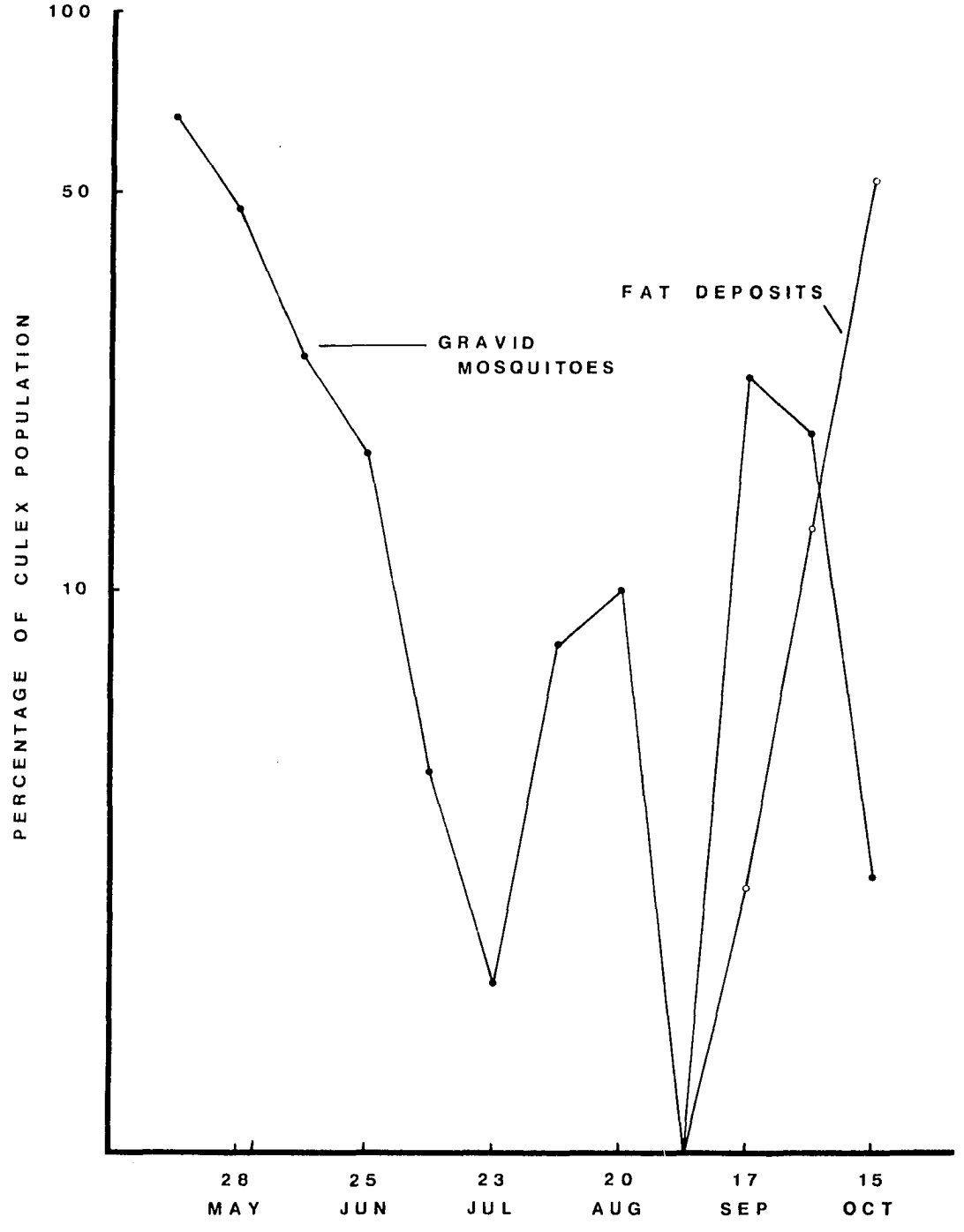
globules were not observed until mid-September in 1978 and the beginning of September in 1979. Culex p. pipiens in the Boston area also did not have fat body deposits until the first half of September (Spielman, 1971; Spielman and Wong, 1973a).

The seasonal pattern of gravid mosquitoes is related to diapause induction because females entering diapause take nectar meals and not blood meals (Clements, 1963). In the present study, gravid Culex mosquitoes are indicative of a digested blood meal since these Culex species were anautogenous.

The seasonal pattern of gravid mosquitoes and females with fat bodies of the diapause-type in 1979 is presented in Figure 12. The inverse relationship that exists between gravid mosquitoes and those in diapause is illustrated by this figure. The number of gravid females decreased in the fall with a corresponding increase in the number of mosquitoes in diapause. The percentage of Culex mosquitoes in diapause increased constantly from September through mid-October at which time trapping operations ceased.

Environmental parameters that effect diapause induction are photoperiod and temperature. Photoperiod at 42°N latitude varies from 13 hr 50 min to 13 hr 5 min from mid-August to 1 September (Beck, 1980). This photoperiod is in the range at which diapause is induced experimentally

Figure 12. Percentage of gravid Culex mosquitoes and female Culex with fat deposits of the overwintering type at Ames, Iowa, 1979


100
50
10
PERCENTAGE OF CULEX POPULATION
GRAVID
MOSQUITOES
FAT DEPOSITS
28
MAY
25
JUN
23
JUL
20
AUG
17
SEP
15
OCT

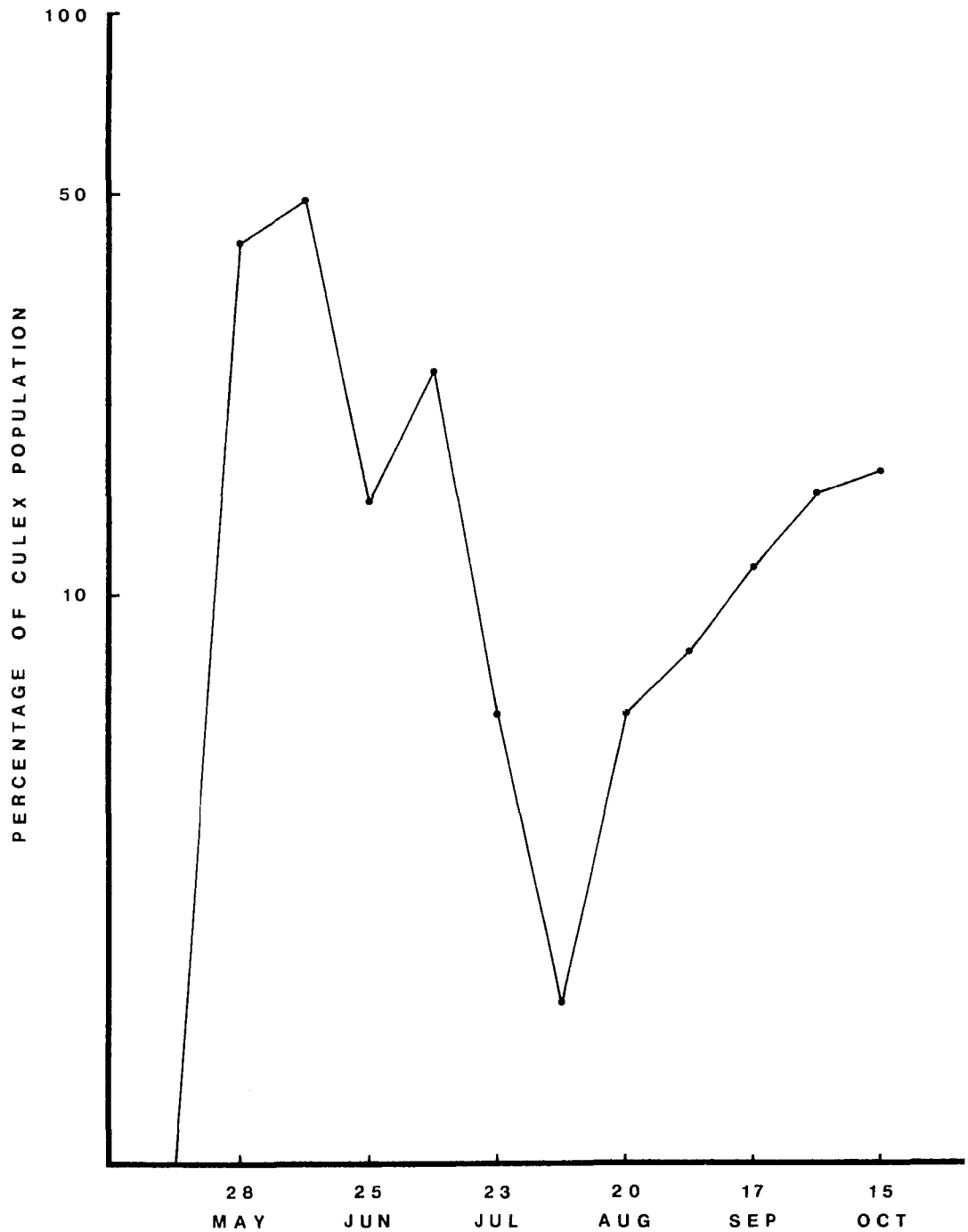
(Eldridge, 1966; Spielman and Wong, 1973b).

Temperature moderates the effect of photoperiod on diapause. Water temperatures below 22°C increase the number of adults entering diapause (Spielman and Wong, 1973b). The mean daily air temperature was below 22°C from 11 to 16 August and again from 23 to 26 August. Only adults that emerge from pupae exposed to temperatures below 22°C would enter diapause. This could explain the decrease in gravid mosquitoes near the end of August. The increase in gravid females in mid-September was probably because the temperature rose from 21 to 25°C from 25 August to 5 September, after which it dropped below 20°C for the remainder of the year.

The influence of temperature on diapause is also illustrated by the pattern of occurrence of gravid mosquitoes in the fall of 1978 (Figure 13). There was a continual increase in the percentage of gravid females into early fall. The difference in the number of gravid mosquitoes in the fall of 1978 and 1979 is probably associated with higher mean air temperatures in 1978. The temperature was above a daily mean of 20°C until 20 September in 1978.

The significance of knowing the period of diapause induction in Culex mosquitoes is related to the role that these mosquitoes play in the natural history of arboviruses and the abatement of epidemics. The occurrence of mosquitoes in diapause at the beginning of September coincides with the

Figure 13. Seasonal abundance of gravid Culex mosquitoes at Ames, Iowa, 1978


100
50
10
PERCENTAGE OF CULEX POPULATION
28
MAY
25
JUN
23
JUL
20
AUG
17
SEP
15
OCT

decrease in the number of cases of encephalitis in past epidemics and epizootics (McGowan et al., 1973; Rowley et al., 1979). The number of mosquitoes blood feeding decreases in the early fall. The result is that virus transmission is disrupted. As the level of virus activity is reduced, epidemic and epizootic virus cycles are terminated.

Another consequence of knowing when mosquitoes are in diapause is related to the implementation of control measures for epidemics. If the majority of the adults are in diapause by late September, it is not economically feasible or realistic from a biological viewpoint to apply an adulticide. These mosquitoes are part of the overwintering population and are not involved in the transmission of arboviruses. Larviciding of Culex mosquitoes from September onward is a waste of money, materials, and man-power because the mosquito population will enter diapause. Therefore, they will not be actively feeding on vertebrates and are of no consequence in the transmission of viruses.

Control measures must be initiated in August, with possible adulticiding at the beginning of September. Environmental temperatures during this period would be important for aiding in the decision of whether or not to implement control measures. If an epidemic seems pending, the application of control measures especially in mid- to late-August could successfully decrease the population level of parous vectors.

Fewer potential vectors would decrease virus activity and the threat of an epidemic.

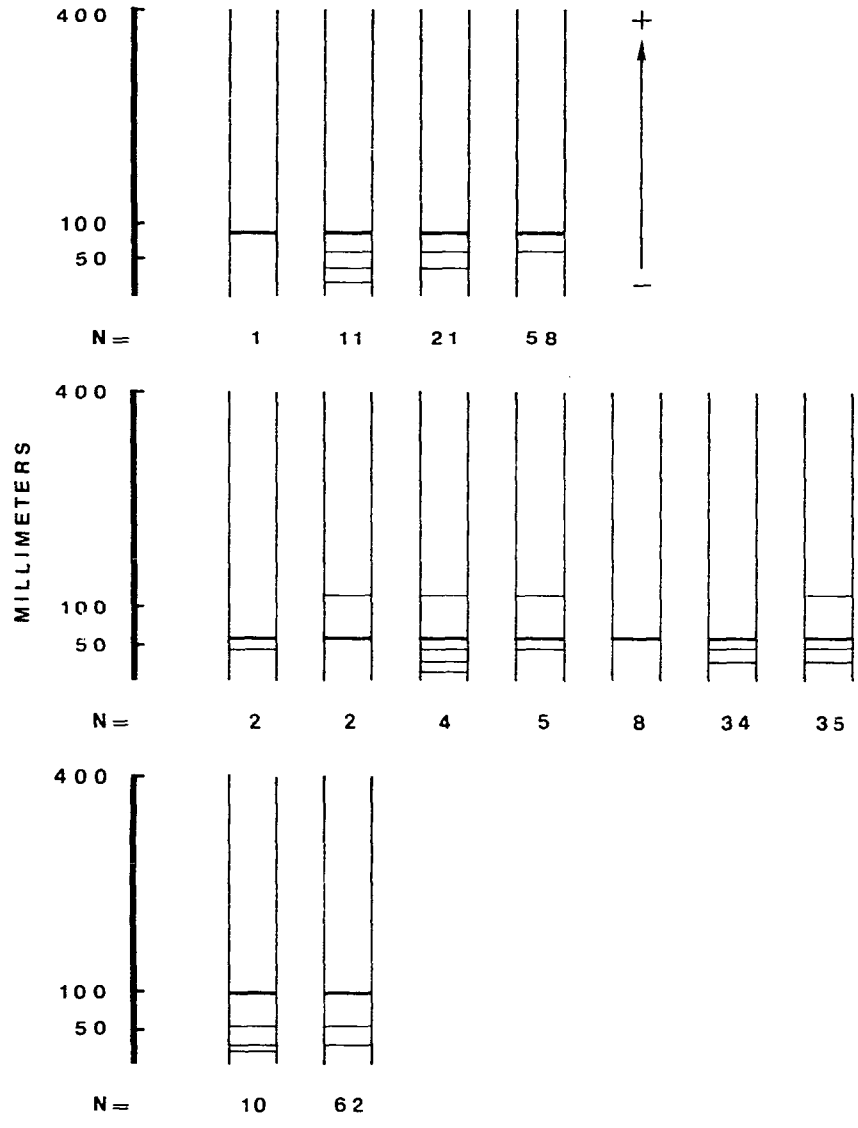
Electrophoretic Identification of *Culex* Species

Adult female *Cx. p. pipiens*, *Cx. restuans*, and *Cx. salinarius* were tested electrophoretically to determine if isozyme patterns of aldehyde dehydrogenase were species distinct. In addition, nulliparous and gravid specimens of each species were examined to determine if the physiological state of the mosquito altered the isozyme pattern for a species.

The numbers of nulliparous *Cx. p. pipiens*, *Cx. restuans*, and *Cx. salinarius* tested were 91, 90, and 72, respectively. The banding patterns of aldehyde dehydrogenase for each species are illustrated in Figure 14. The mean distance each band migrated is indicated in millimeters. Patterns in actual gels for individuals of each species are presented in Figure 15.

Four different staining patterns were detected for *Cx. p. pipiens*. All staining patterns were characterized by one band that was distinctly more densely stained than were the other bands. This concentrated band occurred at approximately the same position on all gels. A maximum of four isozymes was observed. However, 58 out of 91 specimens only had two

Figure 14. Banding patterns of aldehyde dehydrogenase in nulliparous Culex mosquitoes



CULEX PIPIENS PIPIENS L.
(91)

CULEX RESTUANS THEOB.
(90)

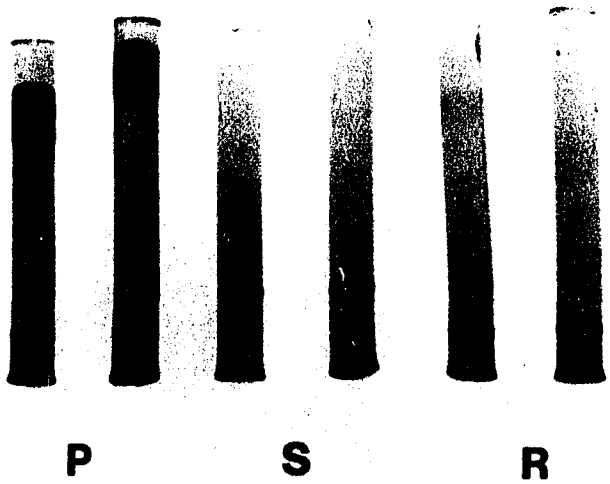
CULEX SALINARIUS COQ.
(72)

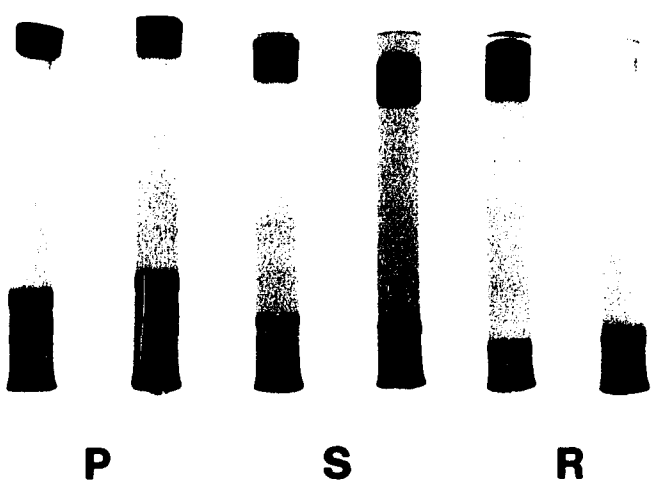
Figure 15. Isozyme patterns of aldehyde dehydrogenase in gels of nulliparous Culex pipiens pipiens L., Culex restuans Theob., and Culex salinarius Coq.

P = Cx. p. pipiens
S = Cx. salinarius
R = Cx. restuans

Figure 16. Banding patterns of aldehyde dehydrogenase in gravid Culex mosquitoes

P = Cx. p. pipiens
S = Cx. salinarius
R = Cx. restuans


P
S
R


P
S
R

isozymes.

There were seven different zymogram patterns observed for Cx. restuans. Predominantly, two patterns were detected. Together, these banding patterns appeared in 77% of the specimens. The number of isozymes recorded varied from one to five. Three or four isozymes were the most common numbers observed. Culex restuans also had one band which was always more concentrated than the other bands. This band, as in Cx. p. pipiens, always occurred in approximately the same place. A fast band was detected beyond the major band in 51% of the Cx. restuans mosquitoes.

Culex salinarius had only two staining patterns. One pattern was detected in 62 out of 72 mosquitoes. This zymogram had three isozymes, but, Cx. salinarius did have four bands in 14% of the females. Again, one band was more densely stained than the other bands and occurred approximately at same location in each gel.

A comparison of zymograms of the three species showed interesting differences between these Culex species. The most striking difference was the consistency in banding patterns of Cx. salinarius. The greatest variability in the number of different patterns occurred in Cx. restuans.

Another important difference is that Cx. restuans has fast bands, while similar fast bands could not be detected in the other species. A third interesting point is concerned

with the staining intensity of the bands below the major band for these species. These bands were distinctly more concentrated in Cx. p. pipiens than in Cx. restuans and Cx. salinarius (Figure 15).

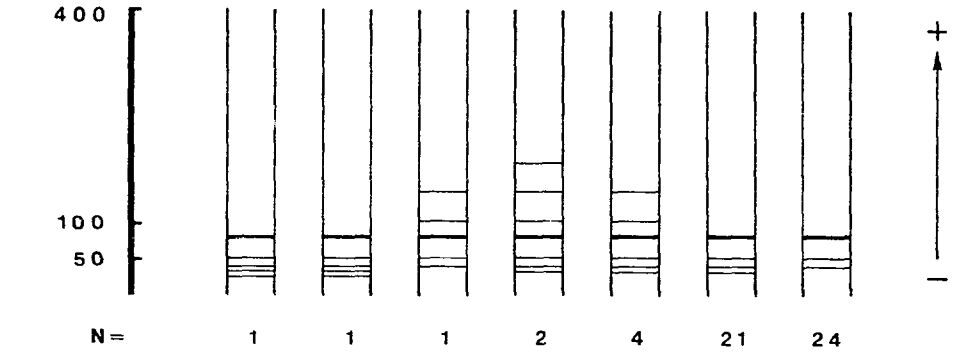
Accurate taxonomic identification of the three species varied according to the species. Fifty percent of the female Cx. restuans tested could be identified accurately by the occurrence of the fast band. Twelve and 14% of the Cx. p. pipiens and Cx. salinarius, respectively, had specific banding patterns that allowed correct identifications.

Isozyme patterns for gravid females of these species are presented in Figure 17. Zymograms of actual gels for representatives of the different species are illustrated in Figure 16. Fifty-four Cx. p. pipiens and Cx. restuans, and 15 Cx. salinarius were tested.

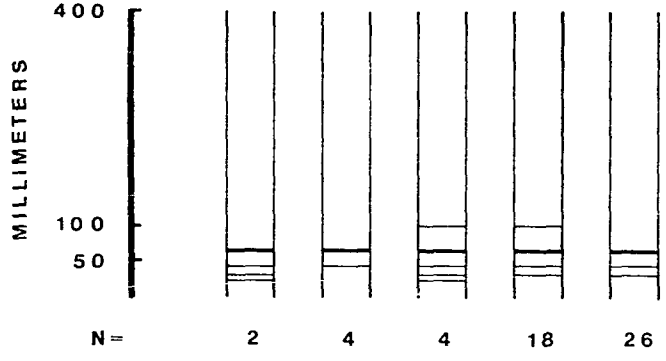
There were seven different banding patterns in gravid Cx. p. pipiens. Two of the patterns were found in 83% of the mosquitoes tested. The number of isozymes ranged from three to seven. Again, there was a concentrated major band in all specimens. Also, two and three fast bands per gel occurred in 9 and 4% of the mosquitoes, respectively.

Culex restuans had five different isozyme patterns. Eighty-one percent of the females were detected by two of the staining patterns. The only difference between the two patterns was the presence or absence of a fast band. A

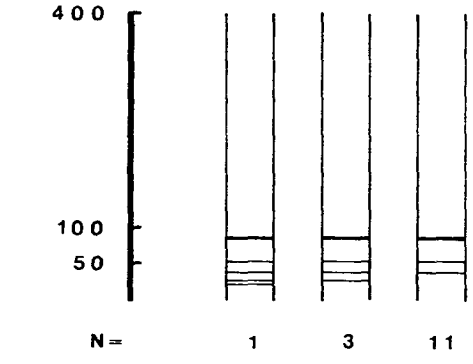
Figure 17. Isozyme patterns of aldehyde dehydrogenase in gels of gravid *Culex pipiens pipiens* L., *Culex restuans* Theob., and *Culex salinarius* Coq.



CULEX PIPIENS PIPIENS L.
(54)



CULEX RESTUANS THEOB.
(54)



CULEX SALINARIUS COQ.
(15)

fast band was observed in 41% of the Cx. restuans. There was a densely-stained major band in all gels. The number of isozymes varied from two to five.

Only three different zymogram patterns were recorded for Cx. salinarius. Eleven out of 15 mosquitoes tested exhibited one particular pattern. This pattern had three isozymes, although up to five bands had been detected. There was also a major band similar to that in the other species.

A comparison of the zymograms of gravid mosquitoes showed some interesting differences. Again, variability of the banding patterns was less with Cx. salinarius. Culex p. pipiens had more patterns than the other two species. Fast bands occurred in both Cx. p. pipiens and Cx. restuans. However, double and triple fast bands were observed in Cx. p. pipiens, while only single fast bands were found in Cx. restuans.

The fast band present in Cx. restuans aided in accurate identification of gravid females of this species. Forty-eight percent of the Cx. restuans were accurately identified. Seventeen and 7% of Cx. p. pipiens and Cx. salinarius, respectively had species specific banding patterns.

Two important distinctions were seen when the zymograms of nulliparous and gravid mosquitoes of the same species were compared. The staining intensity of the bands was greater with gravid mosquitoes than nulliparous ones. This was

especially noticeable with Cx. p. pipiens and Cx. restuans. This relationship did not occur in Cx. salinarius. Fortunato and Fuchs (1980) reported a similar situation with gravid and nongravid Aedes aegypti L. The greater intensity of staining in gravid females is not unexpected because there is an increased concentration of enzymes associated with the presence of eggs.

The other difference between gravid and nulliparous mosquitoes involved the number of detectable isozymes. A maximum of four isozymes was recorded in nulliparous Cx. p. pipiens, while seven were found in the gravid counterparts. Also, 56% of the gravid females had more than three isozymes. Only 12% of the nulliparous Cx. p. pipiens had more than three isozymes. An extra isozyme was detected in gravid Cx. salinarius but no extra bands were found in gravid Cx. restuans. Studies by Geering and Oberlin (1975), and Fortunato and Fuchs (1980) found that the number of isozyme bands in Ae. aegypti increased in gravid individuals versus nongravid females. Fortunato and Fuchs (1980) recorded 6 out of 16 enzymes in which one or more additional bands were detected in gravid mosquitoes.

The significance of this situation is difficult to evaluate. Fortunato and Fuchs (1980) implied that these extra protein forms may be regulated by hormones associated with ovarian development. They cited evidence that the two

new forms of malate dehydrogenase were completely localized in the developing ovaries of gravid Ae. aegypti. In the case at hand, aldehyde dehydrogenase enzyme is involved in converting the amino acid threonine to acetyl-CoA (Lehninger, 1975). Acetyl-CoA is involved in the tricarboxylic acid cycle which is tied to energy production through the electron transport and oxidative phosphorylation chains. Perhaps, the increased demand for energy during egg maturation would account for the differences in some of the observed staining patterns. Different isozymes and/or an increased concentrations of other isozymes may be activated to increase the production of acetyl-CoA.

Two major conclusions are derived from this study. One conclusion is involved with the variability in the staining patterns observed in both nulliparous and gravid mosquitoes. Variability in isozyme patterns is related to genetic variability in the population. Isozymes are formed from information transmitted by genes. Trebatoski and Haynes (1969) recorded isozyme heterogeneity in several species for esterase substrates, acetate and butyrate, and malic dehydrogenase. Many studies since late 1960s have reported a similar phenomenon (Narang and Kitzmiller, 1972; Iqbal et al., 1973; Narang et al., 1977; Miles, 1978).

Agrell and Kjellberg (1965) stated that more than 30 enzymes exhibit multiple molecular forms. The total number

of enzymes with multiple isozymes probably exceeds that number. This great variability in the expression of enzymes can limit its value as a taxonomic "tool" for identification of mosquito species.

The other important conclusion from the electrophoretic study is that the physiological state of a mosquito may influence the banding pattern of an enzyme. Different enzymes and/or concentrations of enzymes are associated with various physiological conditions of insects. Yet, many researchers seem to neglect the effect of the physiological condition of the specimen in their investigation of isozyme patterns. Results of such studies may be in jeopardy of having their interpretations limited to their special conditions. More research needs to be undertaken concerning various physiological conditions (i.e., age of mosquito) in order to determine their effect on enzyme patterns if electrophoresis is to be used as an accurate taxonomic "tool" for species identification of mosquitoes.

SUMMARY

Studies were conducted to determine the seasonal abundance, parity, and the time of diapause induction of Cx. p. pipiens, Cx. restuans, and Cx. salinarius at Ames, Iowa in 1978 and 1979. Also, taxonomic identification of adult females of these species was investigated using polyacrylamide-gel disc electrophoresis.

Seasonal abundance of these Culex species was determined by collecting weekly adult males and egg rafts. Three thousand seven hundred and ninety, and 5,563 adult males and egg rafts were collected in 1978 and 1979, respectively. New Jersey light traps and sweeping trapped numerous male Cx. p. pipiens. More egg rafts than adult males of Cx. restuans and Cx. salinarius were collected. These data indicate that New Jersey light traps, sweeping, and artificial pools are useful monitoring methods for determining the abundance of these Culex species.

Abundance of Cx. restuans was greatest in the spring and early summer. The population peak of Cx. salinarius occurred in the mid-summer while, most of the Cx. p. pipiens were trapped in late summer and early fall.

The seasonal abundance of these species indicated the possible role that the different species may play in natural history of SLE and WEE viruses in Iowa. Culex restuans was

the most abundant Culex species early in the season and may play the more important role in the amplification of virus in natural hosts. Culex p. pipiens and Cx. salinarius were more abundant in late season when epidemics or epizootics of SLE or WEE occur. These species are possible vectors for maintaining the virus in natural hosts and transmitting the virus to dead-end hosts.

Culex restuans and Cx. salinarius readily deposited egg rafts in small bodies of water. Culex p. pipiens showed a "preference" for larger bodies of water. These Culex species may easily become established in urban and rural communities if appropriate breeding habitats exist. Establishment of Culex populations in urban and rural centers could enhance SLE virus transmission in a region especially in an epidemic situation.

The number of adult female Culex collected in 1978 and 1979 was 4,363 and 14,235, respectively. Seasonal parity rates ranged from 19% in 1978 to 47% in 1979. The parity rate was highest in the spring and decreased as the Culex population increased. The maximal number of gonotrophic cycles was two in 1978 and three in 1979.

Parity data support conclusions formed from the seasonal abundance data about the possible roles of these species in the natural history of arboviruses. A majority of Culex mosquitoes had blood fed in the spring and numerous parous

Culex were collected in August.

Diapause induction of adult female Culex occurred the first half of September. All mosquitoes classified as in diapause were nulliparous except for two parous individuals. The overwintering mechanism of SLE and WEE viruses in Iowa does not seem to be related to the overwintering of Culex mosquitoes.

The study on diapause induction showed that ambient temperature has a pronounced effect on the time females enter diapause. A "cool" temperature during late August (daily mean temperature less than 20°C) may prevent occurrences of disease outbreaks by inducing mosquitoes to diapause. The converse is true that a "warm" fall (daily mean temperature greater than 20°C) could extend the time period of an epidemic.

Electrophoretic studies were conducted on nulliparous and gravid females of each Culex species. A maximum of 91 and 54 nulliparous and gravid specimens of each species, respectively, were tested. Fifty, 14, and 12% of nulliparous Cx. restuans, Cx. salinarius, and Cx. p. pipiens, respectively, had species specific enzyme patterns. Similar results were obtained with gravid mosquitoes.

The staining intensity of enzyme bands was greater with gravid than nulliparous mosquitoes. This phenomenon was

probably a reflection of increased concentration of enzymes associated with the presence of eggs.

Five detectable isozymes were observed in nulliparous and gravid Cx. restuans. There was an increase in number of isozymes associated with gravid versus nulliparous mosquitoes in Cx. p. pipiens and Cx. salinarius. A maximum of four isozymes were recorded in nulliparous Cx. p. pipiens while, seven bands were found in gravid females. Only one extra isozyme band was detected in gravid Cx. salinarius. Aldehyde dehydrogenase is tied to energy production. Therefore, the differences in the number and increased concentration of some isozymes may be related to the increased demand for energy during egg maturation.

The studies on the biology of Cx. p. pipiens, Cx. restuans, and Cx. salinarius have shown that these species are potentially important in the natural history of SLE and WEE viruses in Iowa. Environmental temperatures can affect the timing and type (adulticide versus larvicide) of control measures implemented to prevent epidemics.

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APPENDIX: FORMULA

Table A1. Physiological saline for mosquitoes (Hayes, 1953)

Sodium chloride	9.0 g
Calcium chloride	0.2 g
Potassium chloride	0.2 g
Sodium bicarbonate	0.1 g
Distilled water to	1.0 l